

Swords to ploughshares: is it possible?

A NEW initiative is being launched today (December 2) by a group of natural and social scientists to shed some light on public attitudes to disarmament. It originated with M Albert de Smaele, a former Belgian Minister and leading figure in the Belgian Socialist Party, and has been co-sponsored in a personal capacity by a group including Professor V. S. Emelyanov of the Commission on Disarmament Problems of the USSR Soviet Academy of Sciences and Professor Eric Burhop, President of the World Federation of Scientific Workers.

The group rightly notes that it is difficult to arouse public opinion on security questions because of a 'mistrust of opposing theses'. So it tries instead to frame questions on armament and disarmament rather than peddle answers, and it hopes that by getting these questions to politicians, leaders of industry, trade unions, the professions, the universities, churches and so on it might help to get going again a debate which has noticeably run aground in recent years.

The premise of the questionnaire is that either the expenditure of 60% of all research and development funds and the employment of 50 million people in armed forces is futile and a senseless waste, or that sooner or later there will be the 'ultimate catastrophe'. So, run the (somewhat paraphrased) questions:

- can war solve the tensions dividing political systems?
- with the current balance of power, is security better served by competitive escalation of armaments or by balanced reduction—and might some nations in Europe lead the way in a reduction as an example?
- what course is being taken by political thought? Would a debate in Parliament, or in the Interparliamentary Union be appropriate?
- would you favour a collective, controlled commitment to forbid new scientific projects related to mass destruction accompanied by an increase in socially beneficial research; the first consequence of which would be a ban on underground nuclear testing?

● could a military-to-social transition, retaining full employment, work?

● should there be more denuclearised zones, and what further measures might help halt the arms race?

It would be easy to dismiss the questionnaire as naive, if not loaded. The first two questions are really statements—very worthy ones, of course, but calculated to encourage one to dismiss the whole thing as a leftist propaganda exercise and to read no further. This would be a pity because it really would be valuable to hear a wider range of views than is normally advanced on the question of putting military resources, particularly in research and development, to work in new fields.

The huge stumbling block, it is always claimed, is adequate monitoring. There are thirty years of distrust to be overcome—mainly worry by Western nations that the Soviet Union by the nature of its society and its geography, would be able to continue military development clandestinely. Present signs are a little more optimistic: there has recently been revived talk in the Soviet Union about permitting on-site inspection in nuclear test monitoring. Even so, the sponsors of the questionnaire should leave Moscow in no doubt of the disquiet that monitoring questions are bound to raise when it is research and not hardware that is being observed.

It would, nevertheless, be good to get some intelligent non-doctrinaire assessment of what potential there is within the defence industry for a change in purpose. And the first step in that direction is to see whether there are any good ideas around for how a task so financially, politically and technologically complicated could be fulfilled. If the questionnaire did no more than start a modest debate on changing objectives for the 1980s it might have started something worthwhile. For too long military R&D has been regarded as too sacred to touch. □

Fear of flying

Which country gave away the largest percentage of its GNP as foreign aid in 1974?

Which country has legislated that private manufacturing companies offer 49% of their share to employees?

Which airline flies the longest non-stop scheduled flight?

So runs a recent advertisement for Iran Air, which seems also to have a bit of a public relations job to do for Iran. The country is growing fast, thanks to its oil revenues. Its expenditure on arms quadrupled between 1972 and 1975. And because of its military spending and its oil money, it enjoys good international relations with a wide range of nations. Amongst its ambitions is clearly the development of universities and science and technology faculties the equal of those in the West. But amidst all this bustle, some detect a faint smell of the violations of human rights.

Amnesty International, in a briefing which academics might wish to read before visiting the country (40p from 55 Theobald's Road, London WC1), has tried to put together what is known about political prisoners in Iran. Even their numbers are uncertain, ranging from the three thousand that the Shah himself concedes to the tens of thousands claimed by some foreign journalists and exiles. Torture is admitted to by the Shah, and is clearly common, although evidence is not as forthcoming as in some other countries, probably because of the immense power of SAVAK, the security organisation, and the fear it instils. Justice is dispensed with little regard for human rights to those accused of political crimes in Iran. Execution for such crimes is not uncommon. Is it impossible for a rapidly-evolving newly-rich country to survive without such repression? □



Watching the rivers blow

Vera Rich looks at a USSR river diversion project which may use nuclear explosions

A RECENT resolution of the Central Committee of the Communist Party of the Soviet Union and the Council of Ministers on the need for a more rational use of water resources focuses attention once again on one of the most grandiose projects ever proposed by Soviet civil engineers—the diversion of water from the Siberian rivers flowing into the Arctic in order to augment the shrinking resources of the Caspian basin. The growing demands on Soviet agriculture after a series of disastrous harvests and the falling levels of the Caspian and Aral Seas make an injection of new water supplies into this region a prime necessity if the reclaimed virgin lands are not to return to desert and the inland seas are not to become salt-marshes. Accordingly, the final section of the resolution calls for engineering and environmental studies on the practical measures and possible ecological consequences such a plan would entail.

Massive hydroengineering projects are by no means new in Russia. Peter I (the Great) proposed to link the Volga and the Don via their tributaries, the Kamyshin and the Lavla, by a navigation canal; he was thwarted by the local governor, Prince Galitsin, who maintained that “God had made the rivers to go one way and it was presumptuous in man to think to turn them in another”. Peter’s daughter, the Tsaritsa Elizabeth, inaugurated a project to link her country retreat of Tsarskoe Selo to St Petersburg by canal, a scheme which was abandoned half-completed when she died in 1762. The most memorable achievement of the Tsarist era involving water concerned not irrigation but drainage—the reclamation of the Neva marshes and the construction, on this unpromising site, of the new capital of St Petersburg. Indeed, the era left no hydro-engineering works comparable with its last great engineering triumph, the Trans-Siberian Railway.

The new Soviet government soon turned its attention to the possibility of remodelling the waterways. In the 1920s, Lenin’s formula of “Communism=Soviet rule plus electrification of the whole country” initiated a great drive to construct hydroelectric stations. Even during the Revolutionary war, the possibility of building a Volga-Don canal was so widely discussed that it became the

subject of black humour between Stalin and Voroshilov: such major engineering projects, it was felt, would not only be a spectacular achievement for Soviet engineering, with all the attendant publicity at home and abroad; the motif of religion which had frustrated Peter now reappeared in inverted form. The idea was that intervention to “correct a fault” of nature would be a valuable contribution to the atheist re-education campaign because the haphazard and “badly organised” geography which Soviet engineers sought to rectify clearly could not be the work of an intelligent Creator.

It is difficult to establish precisely when the idea of constructing canals for river diversion first arose. The aura surrounding the concept even now is redolent of the atmosphere of the early days of Soviet rule. By the 1950s it had become a desideratum: in one collection of essays on the future of Soviet science published in 1959, it is referred to almost casually in comparison with more modern and daring proposals like the damming of the Bering Straits or the diversion of the North Pacific currents. But the idea of river diversion, couched always in terms of the future, acquired over the years something of a millennial character comparable in a sense to the final achievement of full Communism rather than something likely to be accomplished within the next few Five-Year Plans.

Emphasising the practical

The new resolution, however, although concerned largely with preliminary work, re-emphasises the practical nature of the project and implies that during the current Five-Year Plan considerable efforts and resources will be devoted to feasibility studies and to surveys and comparison of alternative routes. The “classic” rivers proposed for such diversion are the Ob’ and Enisei. According to plans outlined at the International Geographical Congress in Moscow last August a system of reservoirs, pumping stations, canals and tunnels would be built through the Tungai corridor, a depression several hundred kilometers in length. The most promising plan (K. V. Dolgoplov and E. F. Fedorova, *Voda—Natsional’noe Dostoyanie*, Moscow, 1973) envisages a canal from the confluence of two tributaries of the Ob’, the Irtysh and Tobol, which meet at Tobol’sk, to

Atrek, close to the Iranian frontier—a distance of some 2300 km. This would allow up to 50 km³ of water to be directed southwards annually.

The main engineering problem would be posed in crossing the Siberian-Asian watershed, which would involve pumping the water to a height of some 75 m. The cost of the pumping would be considerably reduced by allowing the water to drive hydroelectric turbines on its downward gradient. An announcement at the Moscow Geographical Congress indicates that the canals are also to be of navigable depth, which would favour the ultimate cost-effectiveness of the project. Several giant reservoirs (colloquially called “seas”) with areas up to 100 km² would be constructed along the Ob’, Irtysh, and Tobol rivers to store the water before transmission.

A number of variants of the plan have been worked out. The canal itself could be continued through to Khanty-Mansiisk at the confluence of the Irtysh and Ob’, or else (as Dolgoplov and Fedorova show it), water from the Ob’ could be forced back up the Irtysh to Tobol’sk by a series of pumping stations. The headwaters of the Ob’ and Irtysh are to be joined by a canal already under construction. A sidebranch of the Tobol’sk-Atrek canal could be taken westward over the Urals to feed the Ural river which has insufficient water for the massive irrigation projects planned for its lower reaches.

Such plans have caused considerable outcry among conservationists throughout the world, who fear that a diminution of the inflow into the Arctic Ocean might cause massive environmental changes and even result in a southward spread of the polar icecap. To ecologists not of the doomwatch persuasion, however, such an outcome seems unlikely. The hydrography of the Ob’ basin has been extensively studied. The average annual discharge of the Ob’ at Khanty-Mansiisk is some 233 km³, almost five times the proposed uptake of the canal. At Salekhard, where the river flows into the Ob’ gulf, the annual discharge is 400 km³. The Ob’ basin already has a highly developed economy, including the inland port and industrial complex of Novosibirsk and the vast gas and oil fields of Tyumen’, Urengoi and Samotlor. Hydroelectricity from the lower Ob’ is a fundamental factor in the planned development of these deposits. The Ob’ and its tributaries are, moreover, important fish producers.

As even the plans for the lower Ob’ hydroelectric station were preceded by a detailed survey of the microclimate of

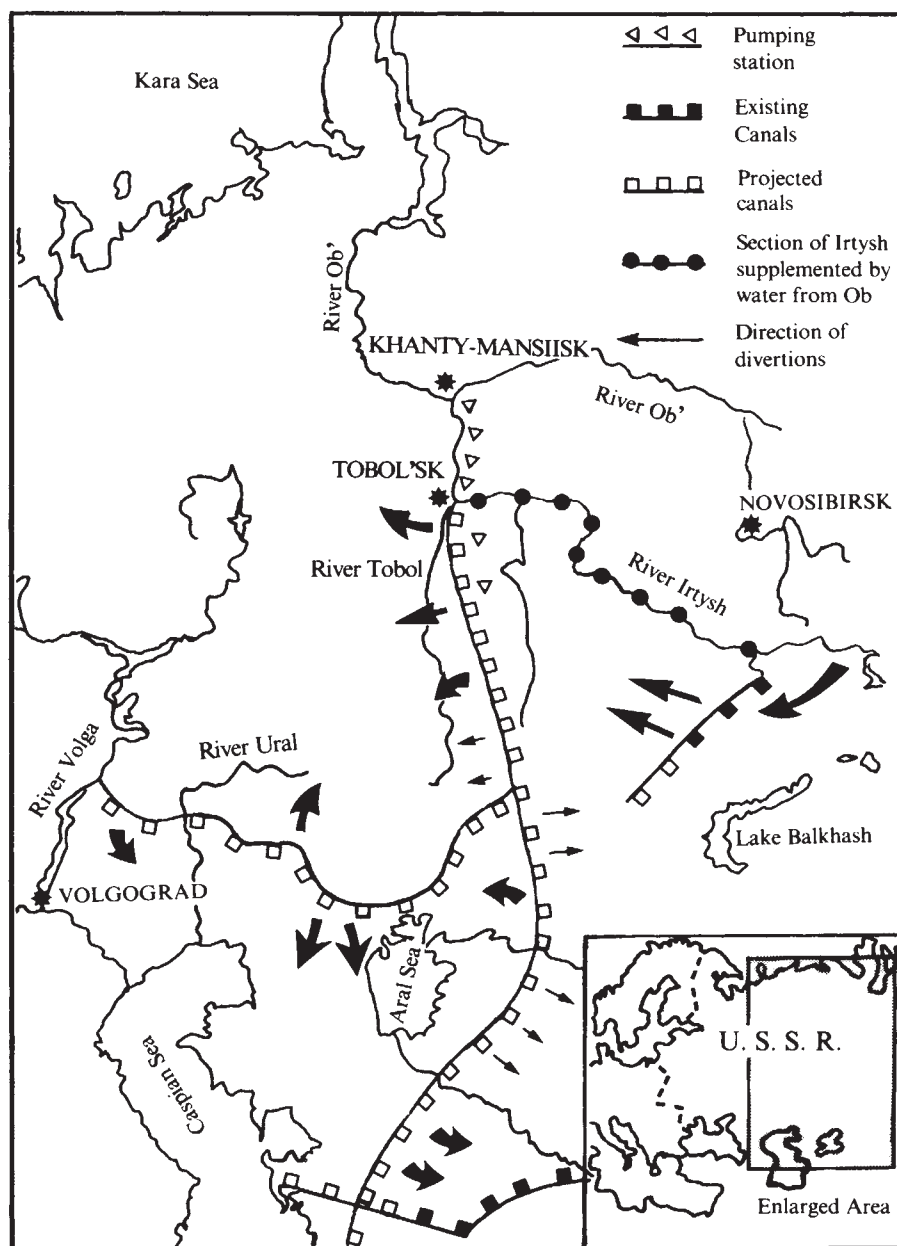
the area, and as the exploitation of the Arctic coast of Siberia is also an important feature of Soviet planning, it would seem highly unlikely that the effect of such proposed diversions would not be investigated as fully as possible. According to the *Novosti* agency, southward redistribution of water from the Irtysh and Ob' (some 75 km³ when all the canals are operational) "would not cause the slightest damage to Siberia". In view of the stress on possible environmental effects written into the resolution this statement may seem somewhat premature, but the emphasis on ecological hazards is significant.

Constructing the canals

Less concern abroad has been evoked by the methods to be used in constructing the canals. Early "spectaculars" of Soviet hydroengineering such as the White Sea Canal were excavated largely by shovel-power and a labour force of "socially undesirable elements" undergoing re-education, but the new canals can command a higher technology—in particular, nuclear explosions. Indeed, such explosions have already been used on another river-diversion scheme west of the Urals which will bring water from the Pechora south to the Kama and thence to the Volga and the Caspian Sea. According to one of the few press articles dealing with this project (it appeared in *Krasnaya Zvezda*, the Red Army newspaper, in 1970), the first stage of canal should be operational by the end of the decade and, with its "cascade" of hydroelectric stations, should recoup all costs within four years.

In view of the fact that, according to Moscow Radio, work on the main canal and reservoirs of the Ob' scheme will not commence until the 1990s, it is interesting that the chief surveyor of the Pechora project, Viktor A. Chistyakov, who had previously worked on the Irtysh-Karaganda canal and the Middle-Enisei hydroelectric station, considered that the terrain of the Pechora project was incomparably more difficult. It is the Pechora diversion which nevertheless seems to have the priority.

The use of nuclear explosives is a feature of the Pechora diggings. The criterion for choosing nuclear rather than conventional blasting is primarily cost-effectiveness; a nuclear charge is more efficient when an explosion of more than a few kilotonnes is required. The Soviet Union has shown an active interest in peaceful nuclear explosions (PNEs) since 1960, when she suggested that a thermonuclear explosion might be used to obtain fresh water from glaciers (the suggestion was not implemented). By using a method of deep-drilling and subsequent collapse, radio-



active products can be confined far below the actual installations, so that there seems to be no great safety-hazard; indeed, a film of an early PNE which formed a crater to be used as a reservoir included shots of a man swimming in the crater only a few days after the blast. (No information was ever released about his subsequent history).

Unlike the United States, which is interested in PNEs principally as a means of gas stimulation and envisages no major construction projects that could possibly employ PNEs, the Soviet Union has in its river-diversion schemes a field where they might play a useful role. It is not surprising, therefore, that the Soviet Union is very interested in the conclusion of a threshold treaty which would define the size and amount of charges to be fired and the conditions under which they may be employed. The proposed treaty, now awaiting

ratification by the US Senate, suggests a maximum charge of 150 kilotonnes, a size which is easy to verify seismologically and which could not be confused with any unauthorised weapon test. If more than one maximum blast is to be used in a single row-charge, a maximum of 1.5 megatonnes is proposed for the row; on-site inspection by the other contracting party would be permitted and, furthermore, that party would be permitted to have monitoring instruments down each borehole to measure the yield. This is the first time that the Soviet Union has expressed a willingness to permit on-site inspection, and may well be an indication of how vital the new canals are to the future Soviet economy. Perhaps the new treaty, with all its political implications, may prove no less a benefit to the canal scheme than the immediate practical gains of irrigation and hydroelectric power. □

At a standstill after a year

Peter Pockley looks at Australian science after 12 months of Mr Fraser's government

NOVEMBER 11, long celebrated in Australia as Remembrance Day to commemorate the end of the First World War, has now been endowed with a second significance. The day was marked in 1976 by large crowds expressing their continued opposition to the manner in which Mr Malcolm Fraser and his Liberal/Country Party coalition persuaded the Governor-General, Sir John Kerr, to sack the Labor Government of Mr Gough Whitlam only two hours after Sir John had laid wreaths on the eleventh hour of the eleventh day of the eleventh month of 1975. Mr Fraser pulled this crisis on Australia, and one month later won the election, on the justification that Labor could not manage the economy.

On his first anniversary in office, Mr Fraser presided over a stagnant economy with his principal target of curbing the 12-14% inflation as much out of range as it was under Labor, with an unemployment rate of about 5% that is the highest since the 1930s and with worse to come as school and university leavers flood the job market in December and January, and with an even worse industrial record than Labor's. Yet his government remains surprisingly popular, a fact which many observers put down to the success with which the release of information and the opportunities for newsmaking debates have been carefully controlled to the point of almost obsessive secrecy. Last weekend's heavy 17½% devaluation of the Australian dollar could dissipate some of that popularity. The Labor Opposition, however, is only just emerging from the shock of its demoralising defeat.

Effect on science

This is the context within which the first Fraser Budget and its effect on science must be assessed. With the one exception of grants for medical research, the effect on science generally has been to impose at least one year of treading water, and quite possibly several years of struggle to stay afloat. Fraser and his Treasurer, Mr Philip Lynch, were determined to tackle inflation principally by drastic cuts in public spending, and their scythe cut deeply into the flesh of all public instrumentalities; unfortunately, most were already lean after cuts by Labor in 1975, and the bones are now exposed.

Take CSIRO, for instance. Its Jubilee year of 1976 has been scarred by a

Budget allocation for 1976-77 of \$131 million (1.6 Australian dollars = £1) which does not even cope with inflation since the 1975-76 allocation was \$123 million. The government claimed the CSIRO's "activities will be held to the same real levels as in 1975-76", a statement greeted with some cynicism by CSIRO administrators as they go about their cheerless task of cutting staff. The foregoing quote from the Budget paper was preceded by confirmation of "an independent external inquiry into the operation of CSIRO". This inquiry is now underway, but as the Budget said, "In the interim CSIRO's activities will be held . . .". It's going to be a hard last period for CSIRO Chairman Sir Robert Price (formerly Dr Jerry Price) and for his unnamed successor who should take over in March 1977.

The 19 universities of Australia are the next greatest sponsors of research. Their growth has been held and there are great uncertainties about the future. Through the Universities Commission, the universities will receive \$562 million for the calendar year 1977, as compared with \$542 million for 1976. The Education Minister, Senator John Carrick, has claimed this constitutes a 2% real growth rate, but no university administrator has publicly agreed; many are trumpeting "hard times ahead". The situation in universities for funding new equipment and replacing old is marginally better than last year, but the real crunch comes in the provision of staff. "Freezing" of establishments and "natural wastage" are the order of the day as universities face unavoidably increasing costs from salary rises as staff grow older on average and are not balanced by equalising intakes of young, cheaper lecturers.

The two principal sources of government money for supporting the research of individuals and small groups, largely in universities, appeared to fare better in the Budget than ever. This was a shrewd, and essentially very cheap, way of the government preventing the kind of damaging outrage which greeted Labor's cuts in these funds in the previous year's Budget. In reality, though, the Australian Research Grants Committee (ARGC) received \$9.1 million, a shade higher only than the previous year. The National Health and Medical Research Council (NH&MRC) was the only group smiling as it received \$9.1 million, a significant increase on \$5.2 million last year; this

Sorry, for copyright reasons some images on this page may not be available online

Mr Fraser, 12 months on

was a tribute to the success of the public lobbying by the medical researchers, which was not matched by those in the natural and social sciences and the humanities serviced by ARGC.

Individual researchers in academia showed last year how well they could protest at threats to their personal support, but in fact they are far more dependent on maintenance of the overall funding received by their institutions and that is decidedly unpredictable. This is because the government has replaced the triennium system of forward funding of universities with, in essence, an annual budgetary arrangement masquerading under the description of "rolling triennium". Significantly also, the previous triennial forward commitments for ARGC and NH&MRC were quietly dropped and only one-year funding substituted.

The Australian Atomic Energy Commission, under its new part-time Chairman, Professor Don George, faces the year with \$21.1 million, radiated up only \$1.1 million on last year. The Antarctic Division of the Department of Science has been frozen at \$6 million. Industrial Research and Development Grants, administered by the Department of Industry and Commerce, have not developed at all—they have dropped by \$3.9 million to \$15.4 million. Labor's Department of the Environment was swallowed into the maw of a ragbag department called Environment, Housing and Community Development with the deliberate intent of spending as little federal money on environment protection and research as possible. The whole department suffered a shattering 37% decrease in funds and the environment part of it did not even rate a mention in the main Budget documents. Defence science and technology received \$89 million (up \$5 million) out of a total defence vote of \$2.178 billion, the only trouble being that nobody really knows what this will be spent on.

Science Department come-back

Reports in these columns earlier this

year chronicled the tribulations of the Department of Science during the first few months of Fraser, when the very continued existence of the Department seemed unlikely. With some good training beneath their skins for survival gained when the Labor Party came to power, the senior staff of the Department knew that if they could survive the first six months of the cost-slashing Liberals they would coast into the period when the new Cabinet aged rapidly as they had to cope with problems of their own making and could no longer blame their predecessors.

This stage has now arrived. But the Department had good reason to be concerned for its future. Its Minister, Senator James Webster, was 25th out of 25 in seniority and his voice was weak in the face of possible Cabinet reshuffles. The Science Task Force of

the Royal Commission on Australian Government Administration had strongly recommended dispersal of the Department's operational divisions among other departments, and there was much support for this view among the scientific community. Fraser had put a hatchet man among the public service to search for and destroy "wasteful and extravagant expenditure", and science was one of those on the block. And there were severe tensions between the Canberra head office and some operational divisions.

In the long run, the hatchets proved blunt and the only waste was the cost of the blades and the unpublished report. The Royal Commission did not come out with a specific recommendation on the Department, and the Secretary, Sir Hugh Ennor, walks taller and looks set to see out his full

term to retirement next year.

Senior members of the Department are setting out to win friends they've not previously had in the universities, at the same time as the Australian Science and Technology Council (ASTEC), a potential rival to the Department in the high stakes of policy formulation, is working hard on its own survival. The Department's data collection outfit, Project SCORE, has its statistics on national R&D expenditure and manpower for 1973-74 at press; official, private inquiries into aspects of research organisation are planned.

The Australian preoccupation with organisations, structures and the dreary politics which go with them to the exclusion of concentration on the substance of research has again been amply demonstrated in Fraser's first year. *Plus ça change* . . . □

CANADA

Reed in the wind

An agreement by the Ontario government to give a paper company the right to cut timber and build a mill in the largest area of the province ever proposed for such a purpose has caused a storm in the provincial legislature. David Spurgeon reports from Ottawa

THE recent controversial agreement between the province of Ontario and Reed Paper Limited covers an area of almost 19,000 square miles, larger than the entire province of Nova Scotia. Although it is subject to the outcome of environmental impact studies and hearings—which, according to the premier, William Davis, will take 2½ years and could still leave the province free to refuse the licence—that has not silenced the critics.

Prominent among them are environmentalists and Indian leaders, and they are led by New Democratic Party leader Stephen Lewis. The "memorandum of agreement" between the province and the company was signed just in time for it to be tabled on the first day of the Ontario legislature's autumn sitting on October 26, and was met by a concerted attack from Mr Lewis. He produced an internal memo from the government's Environment Ministry indicating that some of its own foresters believed the ministry had failed to enforce proper woodcutting practices. The government later said the memo, which warned of a timber shortage by the year 2000, contained only individual foresters' opinions.

The government said the company must submit to a full hearing by the environment board, which heretofore

has dealt only with government projects. All interested parties in the area, such as Indian groups, will be heard by the board. Before that, the company must make public its proposals for a comprehensive forest management plan for its mill, and if the project goes ahead the company must pay back to the government the costs of the studies of timber potential in the area. The agreement expires by January 1980, if the plans are not approved by then.

One of the reasons for the furore is that Reed is the company which owned the chemical plant that was believed to be responsible for mercury discharges into the English-Wabigoon River system, which disrupted life for hundreds of Indians and raised the spectre of Minamata disease among them. The agreement involves some of the same Indians who were previously affected by the mercury poisoning. While those in the area are flatly opposed to the development, which was initiated without consulting them and which would, in the opinion of some, threaten their livelihood, local white residents feel quite differently.

The proposed development would cost about \$400 million and provide up to 1,200 new jobs. It would bring new economic life to the area, whose residents for many years have suffered the uncertainties involved in living in this isolated region. One community is Red Lake (population 2,300), a departure point for rich sportsmen who fly into the area for hunting and fishing. It is also the central housing point for workers in the largest industry in the area: gold mining.

Mr Davis has pointed out that many of those who oppose the Reed develop-

ment have never visited the area and wouldn't know the difference between a balsam and a jack pine. Red Lake Reeve John Goodwillie sees the paper mill as the basis for an economy other than tourism or the vagaries of gold mining. For years the town has seen mines closing and opening, lay-offs and hirings, and market fluctuations. And many agree with him that the mill is a potential boon—not something to be feared. As for the mercury incident, Goodwillie says that as far as he's concerned, "it wasn't Reed that started polluting Dryden, it was a small company and the only thing that Reed did was buy them out and get stuck for cleaning it up."

Ontario's Minister of Natural Resources, Leo Bernier, has in fact been hinting that the company "might just walk away" from the project because of the criticisms. Both Ear Falls and Red Lake councils have passed resolutions supporting the proposed development.

Some see the controversy in a different light. Mr Bernier has admitted, when questioned, that the province is 1½ years behind in its reforestation programme. A paper prepared by an employee of the Natural Resources Ministry raised the spectre of huge acreages going out of production annually as a result of present management practices. It also accused Reed of uncontrolled cutting of Boreal softwoods and partial cutting of high quality stands of Boreal mixed woods.

Public pressure may now force a much more active scrutiny of the province's dealings in many areas, since for the first time a private project in Ontario will have to meet economic tests and numerous environmental, social and cultural tests, too—all of it in public. □

USA

Castles in the air

Colin Norman reports from Washington on a heavily-criticised study from the Environmental Protection Agency (EPA)

A MASSIVE study of the health effects of exposure to low levels of air pollutants, conducted by the EPA at a cost of some \$22 million, is so riddled with errors and shortcomings that the results produced so far are virtually useless. That verdict, reached by a team of investigators working for two Congressional subcommittees, is likely to prove more than a mere embarrassment to EPA, for the study was originally expected to provide the basis for the agency's air pollution regulations.

The study, known as the Community Health and Environmental Surveillance System (CHESS), was an ambitious effort to collect and compare data on the levels of pollutants and the incidence of a variety of health problems in six US cities. Data collection began in the late 1960s and ended last year, and the first results (covering information collected in 1970 and 1971) were published in the form of a monograph in May 1974. The monograph purported to show that there is an association between various health problems, such as the incidence of asthma and heart and lung disorders, and levels of sulphur dioxide in the atmosphere.

Though such a correlation is not exactly surprising—the London smogs of the 1940s and 1950s had provided a rather graphic demonstration of the association between high levels of pollutants and severe health problems—the CHESS findings and the methodology used in the study have encountered considerable scientific criticism.

Most of the criticism has been concerned with the methods used in gathering and interpreting the data, but last February the study was dealt a severe new blow when the *Los Angeles Times* published an article alleging that the finding in the first CHESS monograph had been deliberately distorted by an EPA official "in an effort to prove that pollution from sulphur-bearing fuels has an adverse impact on human health". The article implied that the findings had been distorted in order to provide scientific justification for EPA's air pollution regulations.

The allegations set off a stampede of lobbyists from electricity companies, coal producers and other industries to Capitol Hill, to urge that no new

regulations be adopted at least until the CHESS study has been examined. The matter was urgent because Congress was then about to consider amendments to the Clean Air Act, and consequently two subcommittees conducted a public hearing to examine the charges. The hearing produced a score of witnesses, most of whom flatly denied that any deliberate distortion of the data had taken place. But the hearing did not go deeply into the methodology behind the CHESS study or the validity of the data. For that purpose, a Congressional staff investigation was launched, under the guidance of Congressman George E. Brown Jr, and with the help of a battery of scientific consultants. It was the result of that investigation which was published last week.

The investigating team reported that the CHESS study provides "a picture of a program pressured by EPA management-imposed time constraints to meet legislated mandates for promulgating new standards, hampered by inadequate mechanisms to detect and correct technical problems, and handicapped by budgetary and management restrictions placed on the program after it was well under way". The upshot, Brown said last week, is that the CHESS results published so far "have virtually no quantitative value".

Many of the problems stem from the fact that atmospheric measuring techniques, used particularly in the early stages, are too imprecise to yield good information. For example, an attempt to see whether there is a threshold

level of sulphur dioxide pollution below which health effects are not experienced was hampered by the fact that the measuring technique could not detect concentrations of the pollutant below $25 \mu\text{g m}^{-3}$, and the uncertainty in the measurements sometimes exceeded 100%.

Most of the CHESS data is still to be analysed, but the Congressional report argues that "there is serious doubt that the analysis even when completed will ever be sufficiently credible to support the stated objectives of the program". EPA has, however, initiated a new monitoring program, known as CHAMP (Community Health Air Monitoring Program), which the Congressional report calls "clearly an improvement in aeronomic pollution measurement", but it won't yield health data because at present it is not coupled with a health monitoring programme.

In other words, regulation of air pollution will continue to be based on imprecise information about the effects of low levels of pollutants on human health, a fact which is sure to be exploited to the full by purveyors of sulphur pollutants. They won't have to wait long for an opportunity to state their case, because when Congress returns in January one of its first items of business will be a reconsideration of the Clean Air Act (no agreement was reached last session on proposed amendments to the act, and the matter will be brought up again next session).

It should be noted, however, that although the CHESS study has provided no scientific basis for the present air quality standards, it has provided no reason to reject them. □

Warning on fluorocarbons

Over the shrill objections of the cosmetics industry, the Food and Drug Administration (FDA) and the Consumer Product Safety Commission (CPSC) last week proposed separate actions to reduce and eventually eliminate the use of fluorocarbons in aerosol spray cans. The FDA's proposal, which would involve placing a warning label on fluorocarbon-containing products, was expected; the CPSC action came as a surprise.

FDA has authority over products such as hair sprays, deodorants, perfumes and anti-perspirants, which account for some 80% of the total release of fluorocarbons into the atmosphere. The action announced last week will require the following statement to be printed on the label of aerosol spray cans containing

fluorocarbon propellants:

Warning. Contains a chlorofluorocarbon that may harm the public health and environment by reducing ozone in the upper atmosphere.

No date has yet been set for the regulation to take effect, but barring legal action by the industry to block the proposal, the warning labels will probably be required early next year. The action, noted FDA Commissioner Alexander Schmidt, is only an interim measure—FDA will soon propose a timetable for phasing out all non-essential uses of fluorocarbons entirely.

The CPSC action came in the form of a 5-0 vote by the commissioners to ban use of fluorocarbons in aerosol products not regulated by FDA. No timetable was set by CPSC for carrying out the ban, however.

BRITAIN

Another delay for BNFL

Gillian Boucher reports on the latest developments in the controversy over the reprocessing of nuclear fuel

Just when they seemed about to gel British reprocessing plans have received a vigorous stir from Mr Shore, the Secretary of State for the Environment, who announced last week that planning permission for expansion at Windscale was to be withheld until he had given it more thought. Cumbria County Council had given British Nuclear Fuels Ltd (BNFL) a provisional go-ahead on November 2 but as the application was outside the scope of the county's development plan it had to be referred to Mr Shore.

If he had done nothing the application would have received automatic approval after three weeks. It was agreed at an inter-departmental meeting on November 15 that Mr Shore would not call in the plans. On the last day on which he could intervene he said he needed more time; by last weekend the House of Commons Select Committee on Science and Technology was considering whether to take up the issue and question Mr Shore itself.

Mr Shore has two choices: handing the matter back to the county council, which virtually means accepting it, as the county council was "minded to approve" the application; or calling it in for his own decision, which will probably mean either a public enquiry or a Planning Inquiry Commission and a delay of at least a year.

BNFL wants to expand and update its Magnox reprocessing plant, develop the process of vitrification of nuclear

waste, and build a new "Thorp" plant for reprocessing oxide fuels. It is this last which arouses such passion because of the large amounts of foreign fuel that it would reprocess. Although it means 1,000 new jobs and around £500 million in earnings from deals with Japan, Germany and Sweden already in advanced stages of negotiation, it also carries the dangers inherent in purifying and transporting plutonium. Friends of the Earth feel that the plutonium peril is so great that oxide fuel should not be reprocessed; but a BNFL spokesman says that although oxide fuel is not as susceptible to corrosion as Magnox fuel, corrosion and leaks into the water in storage tanks will eventually occur if the fuel is not reprocessed.

Mr Shore probably "decided not to decide" as a result of strong pressure for a public enquiry from environmentalist groups. But what he wants more time for is not at all clear. He may want to talk to more specialists or learn the views of Mr Carter. Some critics fear that he is merely waiting for the interest built up over recent weeks to die down before giving his verdict.

He has reserved his decision on the whole of the planning proposal, not merely the oxide plant. But the reprocessing of Magnox fuel cannot be delayed and according to BNFL a refusal on that would result in the premature closure of the Magnox nuclear power stations. A decision on the oxide plant is not as urgent because spent oxide fuel can be stored, but Britain already has two oxide-consuming AGR reactors in action. A ban on oxide reprocessing would force Britain

to take its oxide fuels elsewhere for reprocessing and mean the loss of potential foreign business.

Though nobody would deny the gravity of the problems involved, BNFL is understandably irritated that the government should be stalling 8 months after giving it permission to seek foreign deals—permission which was supposed to have been preceded by adequate debate of the issues. And J. C. Stewart, Deputy Chairman of the Nuclear Power Company, said at a British Nuclear Forum Conference last week that it would be "absolutely appalled if the matter did not go forward".

One possible further hurdle, though, lies in the financing of the oxide plant. Last July the government approved BNFL's proposed investment in the Magnox fuel plant and vitrification development, but it has not yet decided whether to act as the lender of last resort for the oxide plant. Most of the finance, however, would come from prepayments from the foreign countries with whom the deals are being negotiated.

Meanwhile the Cumbria council itself may not be allowed quickly to forget its decision. Friends of the Earth and the Lawyers' Ecology Group believe that it behaved improperly in not considering such aspects of the application as the transport of nuclear fuels and the build-up of radioactivity. Mr Shore had written to the council confirming that these were matters for the government, but according to the Lawyers' Ecology Group this does not absolve the county council of its duty to consider the questions. Several pressure groups are likely to come together to take legal action against the council if the planning application is not called in. □

Animal anxieties

BRITAIN'S Medical Research Council (MRC) found it necessary to release a statement last week in an effort to quell a growing storm at its Laboratory Animals Centre at Carshalton, Surrey. The row is over the MRC plan to charge the 70 animal breeders participating in a 26-year-old accreditation scheme the full economic costs of the £70,000 service provided.

The Council said it reaffirmed the importance it attached to its policy, but said it "has been made aware" of the problems in relation to charging for the scheme and had therefore set up a committee with the "necessary powers to act" to review these urgently. It is thought that the committee could report in weeks rather

than months.

Last week, however, delegates from nine trade unions represented at the Carshalton centre called a press conference just as the Council itself was about to meet. They said that if as they expected the Council decided to set up a working party they would have no confidence in this as it was "not a way to proceed". Industrial action was being contemplated, and one union had already asked the Minister of Education and Science to intervene.

The scheme is voluntary and consists of grading, by their degree of freedom from microorganisms, some 3 million animals used every year for research and teaching in labs up and

down the country. The unions say the scheme is now in danger of breaking down totally because the majority of breeders, apparently unable to afford the charges, are threatening mass resignation.

This, they say, would result in "the loss of public control over the commercial breeding of laboratory animals" and could cost the community more than it saves insofar as the use of non-accredited animals grows and leads to costly outbreaks of disease that might not otherwise occur. There is no clear sign, however, that the MRC actually wants the scheme to end, and it seems likely that the new committee will seek ways to maintain it while introducing charges.

Chris Sherwell

IN BRIEF

Environmental warfare action

The Natural Resources Defense Council (NRDC), an environmentalist organisation, has gone to court in an effort to block ratification by the United States of an international treaty prohibiting some types of environmental warfare. NRDC lawyers have argued that since the treaty, which is now being considered by the United Nations General Assembly, would exclude only environmental modification techniques which may have "widespread, long-lasting or severe effects", it would not outlaw use of such techniques as cloud seeding and other potentially more damaging environmental modification efforts. If the treaty is ratified, NRDC asserts, it would serve only to "legitimise the use of weather modification and defoliants as weapons of war".

Similar criticisms have also been ex-

pressed by countries including Japan, Sweden, Pakistan and Yugoslavia. NRDC is seeking a novel way to block US ratification of the treaty: it is arguing that the National Environmental Policy Act requires the State Department to file an environmental impact statement setting out the risks and benefits associated with the treaty, and until such a statement has been published, NRDC is seeking an injunction to prevent US ratification.

Windmill site chosen

A small town in New Mexico has been chosen as the site for the world's largest electricity-generating windmill. The Energy Research and Development Administration (ERDA) last week announced that a 200 kW wind turbine generator will be constructed next year in Clayton, a small town with about 3,000 inhabitants in a windswept

region of the Great Plains. The turbine, which will be field-tested for two years, will be the first wind turbine to be constructed in more than 30 years for generating electricity for public consumption in the United States. It will be followed in the early 1980s by two 1.5 MW generators in other, as yet unannounced, locations. Designs for those two generators are now under development by ERDA and, when constructed, they will be the largest wind generators ever built.

Bomb at Swedish plant

A 25 kg bomb at the Ringhals nuclear power station on Sweden's west coast was discovered last weekend after a Goteborg newspaper received an anonymous letter. The bomb was apparently too far from the reactors to have damaged them but could have damaged power lines and transformers.

THE media are providing news of the furbish lousewort (*Pedicularis* sp.). This is a scrophularious plant that is not yet on the endangered list, but is about to receive its diploma in this category, come 1977. Louseworts were so named in England because it was alleged that "the cattell that pasture where plenty of this grass groweth become full of lice". It seems that about 30 furbish louseworts were discovered in the juggernaut pathway of a new federal electric power dam in a remote corner of northern Maine. Such an event sets up two groups: good guys (lousewort lovers) and villains (power dam protagonists) on opposite sides of a "two-valued orientation". The law does not permit a compromise of moving lousewort clusters to another niche in Maine.

Nostalgically I recall that more than twenty years ago I wrote a short article deploring the march of high tension lines through the wild Ramapo mountains, a home of white-tailed deer, mountain laurel, rattlesnakes and blueberries, only 30 miles north of New York City. Like most protesters, I was then, as now, a great consumer of electric power. I am also a lover of the more common louseworts, whose curly fronds rise in reddish sprouts from the wet mud as soon as the snow melts in high mountain meadows. Later they will unfold their small luminous pink flowers called "elephant heads".

It is important to preserve endangered species, but whether we can preserve their present habitats may be doubtful. Sometimes the niche is at greater risk than the species, which can be transferred elsewhere, as with

some of the noble silva of California. The giant sequoia is indigenous in only a few groves on the western slopes of the Sierra Nevada. Yet its lovers have carried it to distant parts; it towers above native trees on the shores of the Vierwaldstättersee in Switzerland. Specimens of its cones

Endangered species

THOMAS H. JUKES

and branches first reached Europe in 1853, and now two giant sequoias in the garden of a palace in Spain are presumably the tallest trees in Europe. If cherished, they can increase in stature for two thousand years. The coast redwood, a similar species, is probably regarded widely as being endangered, but actually it is only the large specimens that are at risk; the species itself is vigorous and thriving. A two-day hike through the Cali-

fornia coast range near Big Sur impresses one that there are probably, and fortunately, more redwoods (small ones) than people in California.

Surely the skills of horticulturists and plant scientists should be increasingly summoned to preserve endangered plant species. Cloning, asexual propagation and transplanting can all be used. The problems are different for animals. Much praiseworthy effort is currently taking place in zoos to save rare and dwindling species of animals, but, when preserved, where will they live? The inherent behaviour of an individual species towards people may either favour or endanger its survival. In the 1920s, the ornithologist Dawson said that there were 20-odd giant condors left in the USA and that one of their principal enemies was "scientists". If the 1920 figures were correct, the numbers of this bird have not since diminished, but it seems to have lost its ecological niche because of human intrusion, and, perhaps, diminution of food supply. Its smaller relatives, the vultures, are in contrast highly adaptive and proliferative. They even eat carrion on the roads in the form of dead animals killed by highway traffic. But the shy and lonely condor will have none of such contact with the doings of humans.

No regrets have yet been voiced over what we are told is the impending extinction of the smallpox virus. Doubtless this little piece of DNA helped, years ago, to keep the human population curve from ascending too steeply. But admirers of smallpox will find few sympathisers.

news and views

Is HnRNA really pre-mRNA?

from Bob Williamson

THE central dogma (and much experimentation) convinces us that genetic information in animals and plants is encoded in nuclear DNA, copied into messenger RNA and then translated into proteins in the cytoplasm. Therefore there has to be an intermediate, a nuclear RNA molecule (the primary transcript) which includes mRNA sequences and which is processed into the cytoplasm. This idea, postulated and elaborated by Scherrer and Darnell in particular, has been widely accepted for many years, yet is supported by remarkably little evidence.

A large proportion of the RNA isolated from nuclei consists of large molecules some 10 to 20 times the size of mRNA. This is particularly true when pulse-labelled RNA, which has incorporated radioactive precursors for a matter of minutes, is examined. Much of this large nuclear RNA (known for many years as heterogeneous nuclear RNA, or HnRNA) falls apart when denatured, demonstrating that it is nicked and held together by hydrogen bonds—or perhaps is aggregated *in vivo* or during isolation.

The paradox of the rapidly labelled HnRNA is that, as Henry Harris pointed out 15 years ago, the great majority of it is broken down in the nucleus and never reaches the cytoplasm at all. Cytoplasmic mRNA accumulates very slowly, and no one had convincingly demonstrated a precursor-product relationship between HnRNA and mRNA using radioactive labelling. The major problem has been the mixture of different RNA species present in nuclear RNA. Even in erythroblasts, where the protein synthesised is mainly haemoglobin, less than one part in a thousand of the nuclear RNA is globin mRNA—and the nature of the other 99.9% is unknown. Attempts made by Scherrer and John Bishop in particular to assay for specific mRNAs using labelled cDNA demonstrated the presence of globin mRNA in large duck nuclear RNA, but in such small amounts as to raise some doubts as to possible contamination or spurious cross-hybridisation. These doubts were

stimulated by the fact that McKnight and Schimke (*Proc. natn. Acad. Sci. U.S.A.*, **71**, 4327; 1974) in a very careful study, failed to find a nuclear high molecular weight precursor for ovalbumin mRNA, as did Lizardi (*Cell*, **7**, 239; 1976) for silk fibroin gene transcripts. The doubts were summarised recently in a review by Davidson and Britten (*Q. Rev. Biol.*, **48**, 565; 1973).

The most recent study on globin nuclear RNA (in this case from mouse foetal liver) again demonstrates a small amount of "precursor" double the size of the 9S mRNA (Ross, *J. molec. Biol.*, **106**, 403; 1976). The half-life of the precursor is approximately 45 min. No precursor more than double the size of the mRNA was found using cDNA to hybridise across a 98% formamide denaturing gradient. The undoubted experimental care shown by Ross only serves to emphasise that this type of experiment cannot extend our information about HnRNA beyond Scherrer's conclusions of 5 years ago—the "noise" due to other sequences is just too great.

Advances in this field really depend upon the isolation of specific nuclear RNAs containing only a single mRNA or restricted group of mRNAs. With a defined precursor many questions could be answered: the size of the primary transcript, the significance of the many sequence features (oligo(U), oligo(A), double strand regions and palindromes), the arrangement of unique and repetitive transcripts along the length of the HnRNA, and even the position of the mRNA could be studied. (These cannot be determined now because one has no way of knowing whether the average features of the mixture of HnRNAs really relate to specific mRNA precursors or not).

Several groups have been trying to isolate globin-specific nuclear RNA, and the first report to reach print is that of Curtis and Weissmann (*J. molec. Biol.*, **106**, 1061; 1976). They have used a particularly elegant column chromatography technique which was first developed for the isolation of viral nucleic acid sequences,

and should have wide applications for any mRNA or DNA where a large amount of complementary probe is available. The probe in this case was complementary DNA (cDNA) prepared with viral reverse transcriptase, and then elongated with terminal transferase to put on a poly(dC) tail. This behaves just like 'ordinary' cDNA when hybridising to messenger RNA, or the mRNA sequences in nuclear RNA. Therefore, since globin cDNA was used, it identified and paired with globin RNA sequences only, and with the expected kinetics in solution.

The hybrid, of course, now has a poly(dC) tail. This will bind to poly(G) by specific hydrogen bonds. In practice a close relative of G, inosine (I), which has the same pairing properties, was used. With a poly(I)—Sephadex column only elongated cDNA molecules containing poly(dC) were retained. These will, of course, include any that have formed hybrids with globin-specific nuclear RNA molecules.

It was no surprise to find that much of the globin-specific nuclear RNA in the mouse erythroid Friend cell line is approximately the same size as mature, cytoplasmic globin mRNA. However, when RNA pulsed for 20 min was examined, an additional peak at double this size (approximately 500,000 molecular weight) was also found. This had previously been found in duck erythroblasts and mouse foetal liver as well. Again, little material larger than 15S could be isolated after denaturation. Curtis and Weissmann conclude that their results definitively demonstrate a nuclear precursor RNA containing globin mRNA at least double the size of the mRNA itself. A number of controls, using fingerprint analysis and labelled cytoplasmic globin mRNA, demonstrated both the specificity of the method and the absence of aggregation.

The most important aspect of the Curtis/Weissmann experiments, however, is their wide applicability to all animal and plant nucleic acid systems. At the moment, progress is difficult because of the large numbers of genes

and transcribed sequences. There are some 10 million gene-size pieces in the human genome, and at least 1% of these are transcribed in the nuclei of even the most differentiated cell types, albeit at low levels. The affinity chromatography tricks developed here, and similar ones using cDNA covalently bound to cellulose columns (for instance Levy and Aviv, *Proc. natn. Acad. Sci. U.S.A.*, **15**, 1844; 1976), allow the isolation of one specific DNA or RNA sequence from this plethora.

The emphasis during the past few years on covalent integrity of the nuclear RNA (which has led to so much formamide and DMSO technology in an attempt to denature aggregates) does not answer the real point. It would be quite possible for the very large precursor to be nicked during or shortly after transcription, in which case a small mRNA-containing molecule would be obtained. In fact, this obviously does happen, in the course of normal processing to the cytoplasm. It is most instructive to compare these data on isolated RNA molecules with the sizes of primary transcripts seen with the electron microscope.

Charles Laird and his colleagues (Foe, Wilkinson and Laird, *Cell* **9**, 131; 1976) studied electron micrographs of

chromatin-associated fibre arrays of nuclear ribonucleoprotein from milkweed bugs. The non-ribosomal regions of the chromatin had far fewer "growing RNA chains" than the nucleolar regions, but these still could be measured. Each "active transcription unit" has a defined "start point" (showing directly that there is a specific initiation point for non-ribosomal gene transcripts, in itself a finding of some importance). Moreover, the average size of a non-ribosomal RNA primary transcript is approximately 8 million daltons, very nearly the size originally predicted using non-denaturing gradients. Unfortunately, Laird cannot yet distinguish individual mRNA precursors in the electron microscope.

As for so many other problems in molecular biology, DNA recombinants will enable these questions to be resolved. When a genomic DNA sequence containing the globin gene is isolated, it will then be possible to follow the fate during processing of transcripts of the sequences adjacent to, and progressively further from, the coding sequence. Using the Curtis/Weissmann technique in conjunction with a recombinant, true processing kinetics could therefore be studied for the first time leading to greater understanding of control of eukaryotic transcription.

with a mass of about $2.5 \text{ GeV}/c^2$. This is interesting, because it means that we are apparently observing the spectrum of excited states of the charmed particles, both for the baryons in this experiment, and for the mesons in the e^+e^- experiment. Theorists can therefore begin to test quite detailed dynamical models which may shed a great deal of light on the question of quark confinement and the fundamental nature of the strong interaction.

But the most direct probable observation of charm so far comes as a single event reported this week. (Burhop *et al.*, *Phys. Lett.*, in the press). Physicists from ten laboratories in Britain, the USA, Belgium, Switzerland, France and Italy exposed a stack of nuclear emulsion to the neutrino beam at Fermilab. They used spark chambers and counters to identify the possible neutrino events, and followed the particles seen in the spark chambers back through the emulsion to find the primary interaction points. Nuclear emulsions form the tracks of silver grains along the paths of charged particles, and these tracks can be resolved with a precision of around one micrometre. This is much finer than any other particle detector, and it means that the tracks of very short-lived particles can be observed, not just the tracks of their decay products. The photograph is a mosaic of pictures taken through a microscope of the tracks in the interesting event. A number of heavy tracks, and some very light ones can be seen emerging from the neutrino interaction point A, near the left of the picture. One of the lighter tracks can be seen to travel about $180 \mu\text{m}$ and then split at B into three light tracks, with no sign of any heavy recoil. Although one event can never be uniquely identified with complete certainty, by far the most straightforward interpretation of this event is as the production and decay of a charmed particle. The lifetime predicted by the GIM model is a few times 10^{-13} s , which gives a typical decay length of about $100 \mu\text{m}$, very consistent with the event observed. Detailed measurements on the tracks in emulsion show that there must be a missing neutral particle at the decay point, and examination of the spark chamber pictures has shown convincing signs of a K^0 or Λ^0 decay, within the spark chamber array, pointing back very satisfactorily to the event.

This strange particle may well be the only missing neutral, in which case it is possible to estimate the mass of the particle which decayed. Such estimates require further assumptions about the charged tracks from the decay. Are they pions or kaons? Assuming they are pions, the mass of the decaying

Likely examples of charm

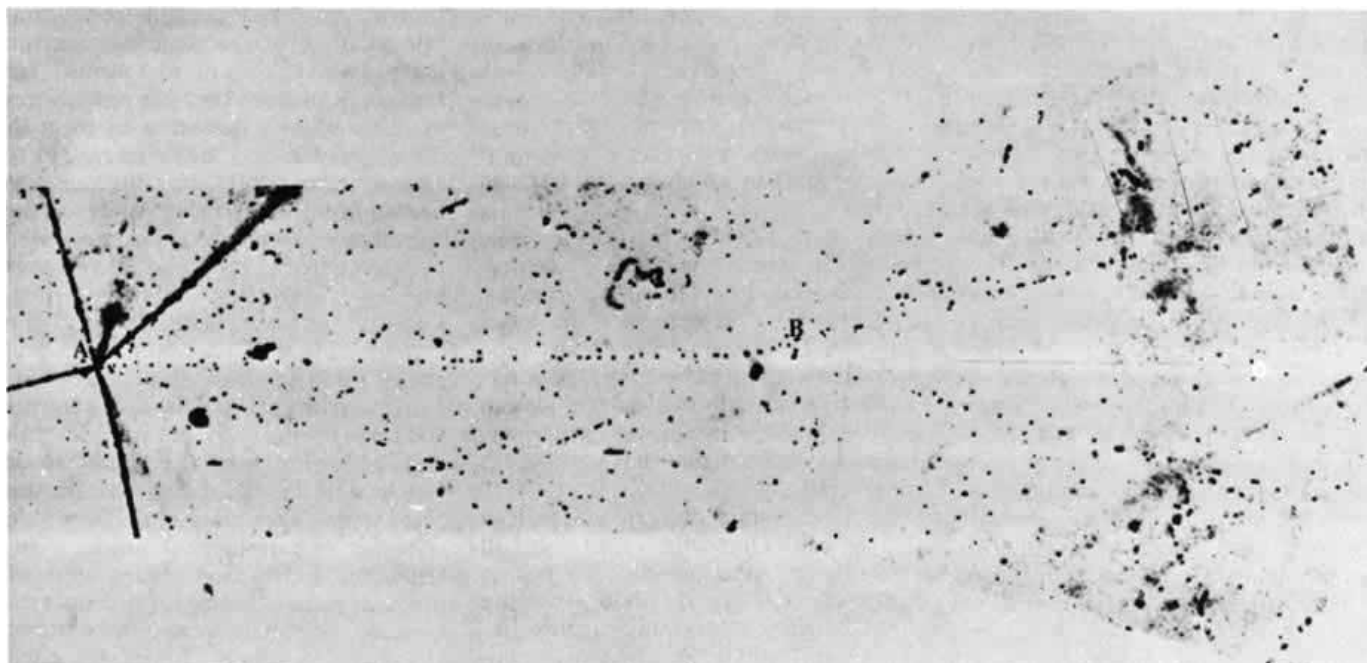
from David Miller

THERE is something very coy about charm. This new quantum number was first invoked to explain the absence of neutral-current processes in the weak decays of strange-particles (see for example, Glashow, Iliopoulos and Maiani, *Phys. Rev.*, **D1**, 1285; 1970; often known as "GIM"). A K^+ meson, for instance, will decay preferentially to a positive muon and a neutrino—a charged lepton system, but a K^0 produces the equivalent neutral lepton system, a positive and a negative muon, with an almost negligible probability. The first experimental evidence for the existence of charm was very indirect; the observation of the new J/ψ family of particles which are now thought to contain "hidden charm" in the form of a quark-antiquark pair. This year the e^+e^- colliding-beam machines have begun to show evidence for the production of pairs of overtly charmed mesons (see for example, Goldhaber *et al.*, *Phys. Rev. Lett.*, **37**, 255; 1976) and neutrino experiments at Fermilab near Chicago and at CERN at Geneva have continued to accumulate events with two leptons and a strange particle in

the final state, which are also usually regarded as examples of charmed particle production (see for example, Benvenuti *et al.*, *Phys. Rev. Lett.*, **34**, 419; 1975; Bleitschau *et al.*, *Phys. Lett.*, **60B**, 207; 1976). Two new results have now appeared to reinforce our belief in the quantum number.

A group from Columbia University, Hawaii, University of Illinois and Fermilab, working in a high energy photon beam at Fermilab, have observed the decay products of a long-lived antibaryon with a mass of $2.26 \text{ GeV}/c^2$. It decays into an antilambda hyperon (Knapp *et al.*, *Phys. Rev. Lett.*, **37**, 882; 1976) and three pions.

The GIM model predicted that the weak decays of charmed particles would produce strange particles, like the antilambda, as a direct consequence of the absence of strangeness-changing neutral currents. Not only does this experiment report the possible "charmed lambda" at $2.25 \text{ GeV}/c^2$, but they see strong indications that it may be produced by way of the decay of a heavier charmed baryon



particle is too low to correspond to the observed charmed mesons (with the neutral taken as a K^0) or the charmed baryons (with the neutral as a Λ^0). If two of them are kaons the mass estimates fit in quite well as either a charmed meson or a baryon. There is no requirement, in any known model, that three strange particles (a lambda and two kaons) should be produced in charmed decays, but there is no reason why it should not happen in perhaps 10 or 20% of events.

This event came from only fifteen neutrino interactions which have so far been identified in the emulsion. The collaboration is still searching for others, but a much larger exposure is planned next year at CERN, where the

new 400 GeV SPS accelerator will be used. Neutrino interactions in the emulsion will be found by projecting back tracks seen in a hydrogen bubble chamber, so the final state particles will be much more clearly identified than they are in the spark chambers. Two or three more events like this one will probably be sufficient to establish, at last, that it is charm that has been discovered. This would not only be satisfying as a vindication of the GIM model; the model is intimately linked with the attempt to unify the theories of the weak and the electromagnetic interaction, so the confirmation of charm will be a sign of real progress in this extremely important programme.

position of the peak response in the array of fibres leaving the cochlea, and intensity by the rate of discharge in the cochlear nerve. The problem arises with stimuli containing several frequencies, because discharge rate has been shown electrophysiologically to saturate at 50 dB, at which level different frequencies should (on position theory) become indistinguishable. But J. P. Wilson and J. Seelman (Keele University) have shown psychophysically that sound level has little effect on the ability of the ear to distinguish the constituents of different sounds (and besides, experience both inside and outside the laboratory amply demonstrates that speech is still intelligible at 70 dB).

Once beyond the auditory periphery, questions of auditory coding begin to overlap with those of visual coding, the central issue being whether single neurones which respond selectively to specific stimulus "features" are a plausible neurophysiological substrate for individual percepts. There have been claims, for instance, that some vertebrate neurones respond specifically to the mating calls of conspecifics.

Those claims, however, are subject to the objections that were raised in connection, for example, with visual "hand-detectors" in the infero-temporal cortex: the methods used simply could not identify such neurones, even if they existed. To resort to *reductio ad absurdum*, on the criteria applied to call-detectors many auditory cortical neurones are white-noise detectors.

More stringent criteria have since been applied by D. Ploog (Max-Planck-Institut) and his colleagues, using the natural calls of squirrel monkeys. Work based on the same principles but with

Sounds and signals

by Miranda Robertson

The Dahlem Conference on the Recognition of Complex Acoustic Signals was held in Berlin on September 27–October 1. A book based on the conference will be published in Spring 1977 and can be obtained from Dr Silke Bernhard, Dahlem Konferenzen, Delbrückstrasse 4c, D-1000 Berlin 33.

IN tackling the issue of how the physical properties of an auditory signal are analysed at various levels of the nervous system to produce a "percept", participants at the meeting were inevitably recapitulating for much of the time arguments that have preoccupied

visual and auditory neurophysiologists for many years and remain unresolved (for an example of the state of the question as visual neurophysiologists left it, see H. B. Barlow, *Perception*, 1, 371; 1972).

But although many of the general questions thus had a somewhat familiar air, some of the specific ones which were addressed exclusively to problems in audition presented a fresh challenge of both theoretical and, often, practical importance.

For example, neural coding is still not properly understood at the auditory periphery, as E. F. Evans (University of Keele) pointed out. It is generally accepted that frequency is coded by the

guinea-fowl was reported at the meeting by H. Scheich (Technische Hochschule Darmstadt), who with G. Langner and D. Bonke has identified the salient features (on behavioural criteria) of several species-specific calls. They tested neurones for selectivity in three ways: with variants of the natural call, with individual salient features of the natural call, and with synthetic calls compiled from those components. They concluded that "call-detectors" consist of neurones with combined selectivity for several call components and which would thus both respond best to natural ("broad-band") stimuli, and tolerate some variation in those stimuli.

An important preoccupation of many of the participants was the identification of the salient features of speech, with a view to the question of whether neurophysiological specialisations exist for speech perception at different levels—for instance, phoneme, word and sentence level. At the level of the phoneme, for example, very small variations in the physical signal can carry enormous linguistic weight. A. M. Liberman (Haskins Laboratories Inc.) and D. B. Pisoni (Indiana University) described work showing that the distinction between "slit" and "split" is made on the basis of the duration of the silence between the "s" sound and the onset of voicing with the "l". "Rabid" is distinguished from "rapid" in a similar way, although other cues (twelve of them) are available to help with that distinction.

At that level, little physiological specialisation seems to be necessary, since chinchillas (which happen to be reasonably sensitive to speech frequencies) do quite well on the rabid/rapid distinction (J. D. Miller, Central Institute for the Deaf, St Louis).

On the other hand, analyses of this kind may be crucial for the understanding of the aetiology of speech problems in children. P. J. Tallal (J. F. Kennedy Institute, Baltimore) reported that the cause of aphasia in some children had been identified as a specific inability to perceive rapidly changing speech sounds—a defect that can be found in adults with certain kinds of temporal lobe lesions.

But while the children can neither understand nor (consequently) produce speech, the lesioned adults can do both. This raises another important point about speech perception. That is that adult speech perception is determined almost as much by experience as it is by the physical speech signal. Pisoni and Liberman pointed out that, for example, coarticulation in normal speech obliterates important cues at the phoneme level, yet speech is still intelligible. That is because the listener already knows a great deal about the structure of language and

(usually) the domain of discourse, and understands the speech stream as much in the light of that as on the basis of its physical properties. Children lack that knowledge and are thus much more dependent on phonetic cues: hence, probably, the difference between Tallal's aphasic children and lesioned adults.

To return to parallels with the visual system, in which the focus of controversy has been how far early experience influences the development of receptive fields, P. D. Eimas (Brown University) suggested that there may be instances in which the auditory system, too, can become biased by early experience. There is evidence that Japanese adults cannot distinguish "ra" from "la" (really); yet Eimas finds that American infants can. Does this mean that all infants can make the distinction, but Japanese ones lose the ability because their language does not require it? Participants pointed out that that question could not be answered until it had been established that Japanese babies could make the relevant distinction, and that the field was fraught with methodological problems because of the nature of the tests on very young infants. A. J. Fourcin (University of London) added that it would be surprising if true, since the evidence says that perception of speech sounds is relatively poor in infants but improves up to the age of 13 or 14.

Plasticity in the auditory system was in fact a topic of extensive discussion, and this report no more does justice to it than it has been able to justice to many other lines of work drawn together by the organising committee under the chairmanship of T. H. Bullock (University of California, San Diego). For reams of fascinating information on echo-location by bats (G. Neuweiler, University of Frankfurt and N. Suga, Washington University), a cogent discussion on whether syntax and categorical coding exist in animal communication (P. R. Marler, S. M. Green, Rockefeller University), and the details of what machines can and cannot "understand" (chiefly M. Schroeder, University of Göttingen) and how to induce them to speak English without a heavy foreign accent—it will be necessary to read the book.

from E. F. Evans

How a scientific study of the biological mechanisms underlying the analysis of complex sounds can aid our understanding of disorders of hearing and language, was the subject of an additional session of the Dahlem Conference on the Recognition of Complex Acoustic Signals. This session devoted most of its time to considering the underlying nature of disorders of hearing and language before moving on to

consider implications for present and future methods of diagnosis and rehabilitation of the deaf.

Because we cannot yet identify all the stages of analysis of speech signals by the auditory system, it is not possible to understand the defective mechanisms responsible for disorders of hearing and language. However, the importance of processing at the peripheral auditory level, and lesions at this level, was stressed. Normal cochlear function is necessary for the correct detection, resolution (in frequency and possibly time), and intensity coding of simple and complex auditory signals, and lesions at this very peripheral level can therefore be responsible for poor speech analysis because of deterioration in frequency response, in peripheral frequency resolution (by which the ear separates out the individual frequency formants of speech), abnormal intensity coding and increased distortion. More centrally, disturbances in temporal analysis (particularly the processing of rapidly changing sounds, including formant transitions) have been shown to underlie speech processing deficits in aphasic children formerly labelled as "language impaired" (Tallal). In other words, the deficits lie in "lower level" acoustic signal processing rather than in "higher level" linguistic domains. Much more work, using the techniques of psychoacoustics and electrophysiology in the laboratory and in the clinic, is required to identify specific defects in speech processing.

It is clear that new strategies in the diagnosis of hearing and language disorders wait upon a more complete understanding. However, new tests are already indicated and are being evaluated. Routine threshold tone audiometry cannot, except in the extreme cases, lead to a prediction of the ability to process speech; on the other hand, the redundancy of the speech signal means that speech audiometry gives little information other than the degree of handicap. New tests are being devised to evaluate the functional state of specific mechanisms of speech analysis, using non-verbal speech-like signals. Thus, tests of peripheral frequency selectivity are being examined; other tests can be devised to evaluate both peripheral frequency selectivity and central pattern recognition (J. L. Goldstein, Tel Aviv University); and a battery of tests is in use to investigate systematically the normality of temporal and spectral analysing mechanisms in patients having impairment of language acquisition in spite of normal pure tone audiometric thresholds (Tallal).

As far as rehabilitation was concerned, great interest was shown in the recent attempts to preprocess speech

signals for patients with peripheral hearing losses, particularly those using filtering and frequency-specific compression, or dichotic presentation of electronically separated formants (W. A. Ainsworth, Keele University). Preliminary results were encouraging, but it is too early to predict the practical outcome for the deaf. Attempts to provide the profoundly deaf with speech information through visual and tactile inputs have proved disappointing, although it is possible to introduce prosodic information such as intonation (important for interpreting the meaning of speech signals) successfully through a multi-channel vibrator prosthesis (A. Risberg, Stockholm University). The restrictions on the rate of information processing presented by the relatively low temporal resolution of the skin and retina indicate that electrical stimulation of the auditory system, for example, by cochlear prostheses, may offer the most likely chance of useful rehabilitation of the profoundly deaf not helped by hearing aids. Present work with single electrode cochlear prostheses is encouraging, particularly for imparting prosodic information, such as the fundamental frequency of speech as indicated by A. J. Fourcin (University College, London). In this way, it ought also to be possible to signal specific speech features such as the "voiced-voiceless" distinction. Both of these should constitute an invaluable aid to lip-reading. However, the full range of speech features required for complete intelligibility can probably only be imparted by multi-electrode stimulation with adequate electrical isolation of the electrodes. This does not seem to be realisable in the foreseeable future.

Honey in the tropics

from a Correspondent

The First Conference on Apiculture in Tropical Climates, was held in London on October 18-20. It was arranged by the International Bee Research Association, in conjunction with the journal *World Crops*. The Conference ended by framing 23 resolutions for submission to international and governmental agencies. A copy is available from the International Bee Research Association (Hill House, Gerrards Cross, Bucks SL9 0NR), together with details of the published proceedings of the Conference. A Second Conference on Apiculture in Tropical Climates is proposed for 1979 or earlier, if possible in the tropics.

THE programme at this first conference ranged over topics as diverse as the

chemical nature of bee sex pheromones and the state of development of bee-keeping in Ethiopia. Tropical countries that want to increase their honey production are understandably tempted to import exotic races, strains, subspecies or species of honeybee that reputedly store honey faster and in larger amounts, but several speakers emphasised the difficulties, and indeed the dangers, of doing this. The aftermath of the 1956 importation of a few queens of the tropical African honeybee *Apis mellifera adansonii* into South America is common knowledge.

The European honeybee *Apis mellifera*, an excellent honey producer in many temperate parts of the world, has now been imported into various countries of tropical Asia where the native *Apis cerana* is not very productive. Some of the importations have been inexplicably disappointing. Soekiman Atmosoedaryo reported on a recent introduction of *Apis mellifera* into Indonesia by the Forest State Corporation, of which he is Director. Colonies had dwindled away and died, in a way that was only too familiar to other Conference delegates. Possible reasons include the lack of adaptation of *Apis mellifera* to the many natural enemies of honeybees in the tropics—including other insects, and birds and mites. Also this temperate-zone bee follows a diurnal foraging pattern that is ill adapted to tropical plants presenting their nectar and pollen only in early morning and late afternoon.

N. Koeniger (University of Frankfurt, Germany) reported research that throws light on another difficulty experienced by imported species—that of mating. Queens and drones of any *Apis* species fly during a specific period of the day. If one *Apis* species is introduced into the territory of another, whose queens and drones fly during a period overlapping that of the imported species, the imported queens are unlikely to mate. Local queens, which are far more numerous, produce the same sex attractant as imported queens, 9-oxodecenoic acid, so drones of both species chase them, but only the local drones can mate with them. Most of the drones chasing an imported queen will also be of the local species; they cannot mate with her even if they locate her, but they keep away imported drones which could mate.

Apis mellifera and *Apis cerana* are not known to coexist in nature; if they do so, it would seem likely that their mating flight periods have become differentiated, as Koeniger found with the three *Apis* species present in Sri Lanka.

The management of bees for honey and wax production poses very



A hundred years ago

The directors of the Swedish Government railways have turned their special attention to the frequent occurrence of colour-blindness amongst their engine-drivers and other officials. Prof. Holmgren has lately examined the whole staff of the Upsala-Gefle Railway, and amongst the 266 persons examined has found no less than eighteen who suffered from this defect, and who therefore were utterly useless and unfit for railway service.

A very fine new university building has been erected at Kiel, one marked peculiarity of which is that it has no "carcer", or prison, which hitherto, it seems, has been an invariable appendage to German universities.

From *Nature*, 15, November 30, 110; 1876.

different problems in tropical and in temperate climates. Most of the early studies on bees were made in temperate regions, and perhaps for this reason, bee behaviour there has often been regarded as normal, and behaviour in the tropics as aberrant; yet it may well be the other way round. When the food resources of an area fail, tropical honeybees commonly migrate to an area where forage exists, as entire colonies, leaving their established nests (T. M. Chandler, International Development Research Centre, Nairobi). As a means of survival this migration is an alternative to the massive food hoarding of honeybees which have to survive a winter dearth period.

Jim Nightingale (Sasumua Estates, Njoro, Kenya) has lived with bees in Kenya for 54 years, and he was able to describe bees' migration routes in earlier days when human use of land was sparse and primitive: to-and-fro movements between the highlands and the floor of the Rift Valley—between the Aberdare Mountains and Naivasha, between the Mau forests and the Valley, and between the Kipsigis areas and down towards Lake Victoria. The human population has now upset age-old patterns in Kenya, but Ethiopia is not yet changed. Mammo Gebreyesus (PO Box 30407, Addis Ababa) maintains apiaries in the highlands, and in the Rift Valley 55 km to the east and 1,500 m lower down. He achieves continuous honey production by moving his hives between the two apiaries at the migration periods, so that the colonies do not leave them.

Mammo Gebreyesus is one of Ethiopia's very few modern beekeepers:

of around one million beekeepers only thirty use modern hives. The fact that the country has three million primitive hives exemplifies the scope for modernisation of beekeeping in the tropics. But, in addition, many areas in tropical Africa, Asia and America are grossly underpopulated with honeybees, and nectar and pollen resources go to waste.

After the sessions on bees, bee management and handling bee products (especially honey and beeswax), came the final session on apicultural development programmes, and this could usefully have lasted two days instead of four hours. It was the first meeting between representatives of donor agencies (from four continents), specialists in charge of their beekeeping programmes, and counterpart specialists from developing countries. At least 53 beekeeping development programmes have been or are now in operation. Tecwyn Jones (Centre for Overseas Pest Research, Ministry of Overseas Development, UK), who opened the Conference, stressed the advantages of beekeeping as a family-level exercise for the peasant farmer. It requires little financial input, and only a few square metres of land; it can be undertaken by people of almost any age, and the commitment can be variable, from a minimal spare-time one to full-time professional occupation. Even spare-time, it may well provide a peasant farmer with a much larger cash income than his usual full-time farming gives him. And it is environmentally sound; nectar and pollen are rarely utilised to the full unless there are social bees to take them, and beekeeping does not compete with other agricultural activities. Indeed it can greatly enrich them, through the bees' pollination of crops. □

Boats from the past

A Symposium on Boat Archaeology was held at the National Maritime Museum on September 20–24, 1976.

from Lynette Hamblin

BOATS and ships are among the largest and most complex movable structures attempted by man throughout his history, and as such offer much insight into his technical capability and organisation throughout the ages. Boat archaeology, however, is still in its adolescence and the symposium brought together nearly 100 international experts to discuss a number of the basic disciplines relevant to this specialised branch of archaeology. Techniques for recording finds and

presenting information, the problems of building boat replicas, hypothetical reconstructions of boat finds and ancient boatbuilding methods were all covered.

In-depth field recording is essential in any archaeological excavation, particularly in the case of wreck sites underwater, where material which has been perfectly preserved is prone to rapid destruction once on dry land. Whereas objects can be lifted and preserved without overwhelming difficulty, the timber of a ship presents a different problem. Lifting any boat can overstrain the conservation resources of a national museum and, unless special units can be set up to deal with specific craft, it is impracticable to haul hundreds of feet of waterlogged timber out of their protecting environment into an atmosphere where they will quickly twist, warp and ultimately disintegrate if not treated. The great ship of the Egyptian Pharaoh Cheops was preserved for 4,500 years in a bone dry, hermetically sealed pit; in the mere six years since it has been excavated and reconstructed condensation and lack of climatic stability has set up drastic deterioration. The air conditioning in the glass-walled museum that houses it is inadequate and the stress on the rebuilt timbers is proving critical.

As naval architect J. F. Coates pointed out boat reconstructions present many problems. Very often a boat find consists of only the bottom of the hull and one, if not both, ends may be missing too.

The Bronze Age boats found at North Ferriby present reconstructors with many puzzles. Nearly all the main pieces forming the bottom, and a bit of one end of a lower side strake were found; these were massive pieces of hewn oak sewn together with yew twig stitches. Coates described three possible reconstructions based on this material. The first was an effort to design a boat with the smallest amount of extra material in it. Such a boat could ferry ten people and eight paddlers across the Humber. The second reconstruction was a more ambitious and higher quality craft which could carry a larger load. However, strains increase with load and weight so design number two may be asking too much of its moss caulking—it would be necessary to find out how badly the boats leaked and this calls for a replica. His third reconstruction moved away from the actual remains. He combined the very heavy oak pieces forming the lower shell with a much lighter skin construction for the upper parts. The adze marks on the North Ferriby boats are of the maximum size possible with the available tools in-

dicating that these boats could have been the Concordes of their time—"in proportion to the Gross Tribal Product they could have been as expensive but arguably more useful".

However, no matter how well a reconstruction is worked out by theory and applied knowledge no naval architect would be too confident of a boat's practical performance unless a replica had actually been made and tested at sea. The National Maritime Museum has pioneered this form of experimental archaeology in its practical research with a replica of the Gokstad *Faering*. Line drawing and a construction plan of the Oslo *faering* were published by A. E. Christensen in 1966 and these were of use in building the replica. However, it was only once the *faering* had been rebuilt and was undergoing sea trials that it was realised why the Oslo *faering* had such large holes in the forward *stammering*—the holes in the NMM *faering* were too small for a man's hand making it difficult to haul the boat up the beach.

Experimental archaeology is concerned with the processes and production methods used in the past, and with the function of the artefact. The reconstruction of an ocean-going skin boat, the Brendan exemplifies the many factors that need to be considered. This type of boat may have been used in the west of Ireland between the 5th and 9th centuries AD. Evidence for their design was gathered from references to skin boats in contemporary texts, ethnographic evidence of the skin boats descendants, surviving examples of leather boats (all river craft), materials research as well as research into the contemporary techniques available for leather and timber working.

With the help of the British Leather Manufacturers' Research Association a test programme was conducted into the properties of various leathers. It was found that by far the most suitable leather for the hull covering was the mediaeval type of leather, tanned in oak bark. A naval architect produced the plans for the replica and shipwrights were asked to fair up a wooden frame to these drawings. Only timber available in Ireland in the early Christian era was used: oak for the gunwales and thwarts, ash for the frames and stringers. The frame was lashed together using alum-tanned leather—a technique known in Roman times—and the entire structure was coated with wool grease to preserve the ash frame and protect the leather. Forty-two ox hides were needed to cover the boat, stitched together with hand-rolled flax. The Brendan has now successfully sailed to Greenland where it will have to spend the winter before completing the trip to North America.

articles

Implications of pollen assemblage from the Koobi Fora Formation, East Rudolf, Kenya

Raymonde Bonnefille

Laboratoire de Géologie du Quaternaire, CNRS, 92190, Meudon-Bellevue, France

A pollen spectrum obtained from the Ileret Member of the Koobi Fora Formation indicates that vegetation patterns differed significantly from those prevailing east of Lake Rudolf today. The pollen-bearing sediment, which is interstratified with Plio-Pleistocene hominid fossils, shows somewhat more humid conditions.

PLIO-PLEISTOCENE lacustrine and fluvial strata exposed along the eastern side of Lake Turkana (Rudolf), Kenya are notable for their rich assemblages of fossil vertebrates¹, hominid remains² and early artefacts³. The geology and stratigraphy have been described by various authors who have worked in this area since 1969 (see refs 4-6).

Preliminary samplings for pollen analysis were taken at different localities where outcrops of the Koobi Fora Formation are exposed. Of the 27 samples, collected and examined, only one yielded pollen in sufficient quantity to justify study and interpretation. The pollen assemblage, Koobi Fora Formation Pollen Spectrum No. 1 (KFFP 1), was extracted from sandstone 3 m above the hominid site KNMER 1592 at Ileret, area 12 (Fig. 1). It comes from a stratigraphic level situated between the KBS Tuff and the Lower/Middle tuff complex. Two different sets of dates have been proposed for the KBS Tuff, one⁷ assigning it an age of 2.6 ± 0.26 Myr and the other assigning⁸ an age of 1.6 ± 0.05 Myr to the Upper KBS Tuff and 1.82 ± 0.04 Myr to the lower tuff.

The Ileret Lower/Middle Tuff complex has been dated⁷ at 1.48 ± 0.17 Myr. The pollen sample comes from just above the level which is interpreted equivalent in time to the post erosion surface (I. Findlater, personal communication) at an horizon that shows reverse polarity in other sections. From this it follows that if the first hypothesis is correct, the stratigraphical level from which the pollen assemblage was extracted would be dated between 1.8 and 2.4 Myr. For the time being it seems reasonable to treat the age of the pollen assemblage as being $\sim 1.5-1.6$ Myr.

Pollen extraction was undertaken on a 100-g sample of sediment after a positive result had been obtained with a 20-g sample. The chemical process involves first an attack with a 10% solution of HCl, dissolving the silicates with a 70% solution of HF, and subsequent density separation of the organic matter using 'Liqueur de Thoulet'. All the residue has been observed. The total count of pollen (696) represents the absolute quantity recorded in 100 g of sediment. No heavily corroded pollen grains were noticed.

Palynological results

Details of the pollen assemblage are given in Table 1. Identifications of pollen grains are based on a reference

slide collection which contains $\sim 4,000$ East African species. Morphological studies of > 350 species from the Omo area including light and scanning electron microscopic observations were also used. As can be seen in Table 1, some taxa have been identified at the family level, others at the generic or specific level.

Fig. 1 Map of the East Rudolph region, showing the sampling areas.

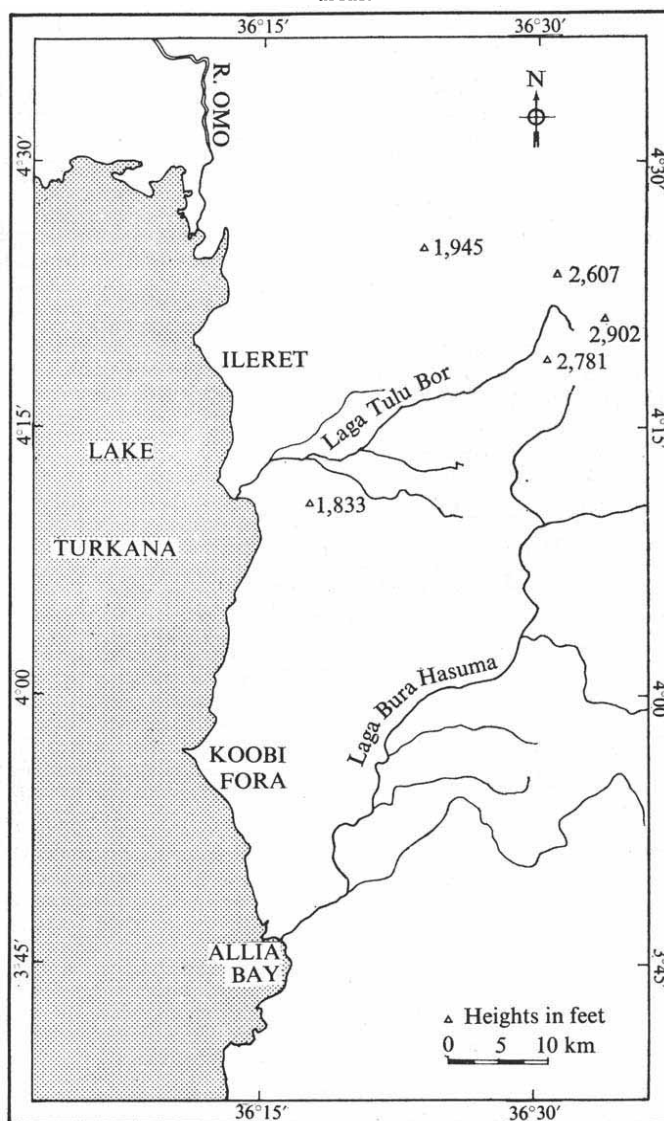


Table 1 Fossil pollen spectrum of a sample from the Koobi Fora Formation (Ileret, East Rudolf, Kenya), as percentage of the total

		No.	%
(1) Taxa not represented in the East Rudolf area*			
A Taxa present in the Ethiopian Highlands			
Ericaceae		2	0.2
Podocarpaceae	<i>Podocarpus</i>	19	2.7
Cupressaceae	<i>Juniperus</i>	26	3.7
Oleaceae	<i>Olea</i> cf. <i>O. hochstetteri</i>	1	0.1
Rosaceae	<i>Hagenia abyssinica</i>	4	0.5
Sapindaceae	<i>Dodonaea viscosa</i>	8	1.1
Euphorbiaceae	cf. <i>Croton</i>	1	0.1
Myricaceae	<i>Myrica</i>	1	0.1
Sp. A cf.	<i>Bosqueia</i>	16	2.2
Sp. B cf.	Meliaceae	3	0.4
Compositae liguliflorae		1	0.1
Compositae	<i>Artemisia</i>	1	0.1
Acanthaceae	<i>Mimulopsis</i>	1	0.1
Plantaginaceae	<i>Plantago</i>	4	0.5
Urticaceae	<i>Droguetia</i>	4	0.5
Caryophyllaceae		3	0.4
	Subtotal:	95	13.6%
B Taxa present in riverine vegetation near the Omo river			
Anacardiaceae	<i>Rhus</i> sp.	2	0.2
Moraceae	<i>Ficus</i>	1	0.1
Euphorbiaceae	<i>Ricinus communis</i>	1	0.1
Celastraceae	<i>Maytenus</i>	3	0.4
Combretaceae	<i>Combretum</i>	2	0.2
Palmae	<i>Hyphaene</i>	1	0.1
Amaranthaceae	<i>Celosia argentea</i>	2	0.2
Amaranthaceae	<i>Celosia</i> cf. <i>trigyna</i>	3	0.4
Acanthaceae	<i>Justicia anselliana</i>	3	0.4
	Subtotal :	18	2.5%
	A + B =		16.2%
(2) Taxa present in the East Rudolf area			
Capparaceae	<i>Cadaba</i>	5	0.7
Capparaceae	<i>Boscia</i>	1	0.1
Mimosaceae	<i>Acacia</i> (2 pollen types)	18	2.5
Burseraceae	<i>Commiphora</i>	5	0.7
Salvadoraceae		37	5.3
Amaranthaceae	<i>Sericocomopsis</i>	1	0.1
Tiliaceae	cf. <i>Grewia</i>	1	0.1
Acanthaceae	<i>Justicia</i> cf. <i>odora</i>	1	0.1
Compositae	cf. <i>Pluchea</i>	1	0.1
Chenopodiaceae		30	4.3
Amaranthaceae	<i>Digera</i>	3	0.4
Euphorbiaceae	<i>Euphorbia</i>	35	5.0
Solanaceae	<i>Solanum</i>	1	0.1
Nyctaginaceae	<i>Commicarpus</i>	1	0.1
Acanthaceae	<i>Blepharis</i>	1	0.1
Liliaceae		3	0.4
	Subtotal:	144	20.6%
(3) Ubiquitous taxa			
Compositae tubuliflorae		14	2.0
Acanthaceae		2	0.2
Gramineae		303	43.5
Typhaceae	<i>Typha</i>	17	2.4
Cyperaceae		75	10.7
	Subtotal:	411	59.6%
(4) Pteridophytes			
		15	2.1%
(5) Unknown			
		13	1.8%
	Total =	696	
Spores Anthocerotales: 12			

*Not recorded in the checklists established by J. B. Gillett, East African Herbarium (1971, 1974).

Taxonomic identifications

Taxa identified as Gramineae, Cyperaceae, Compositae, Liliaceae, Caryophyllaceae belong to botanical families in which pollen grains are very homogeneous. Pollens of grasses are porate, with one pore and a smooth exine. They offer very few characteristics which could be used for generic diagnosis. In the fossil pollen assemblage, grasses are very abundant, representing 43.5% of the total count. This percentage includes grasses growing near the lake shores or river banks as well as grasses in the savanna which cannot be distinguished on the basis of diameter measurements. Other taxa identified as Ericaceae, Acanthaceae, Liliaceae represent fossil pollen grains in which some details are not preserved, preventing identification beyond the family level.

Generic and specific identifications of the Amaranth pollen grains are based on recent work by Rioulet and Bonnefille⁸. Of the genus *Olea*, six species are known in the East African flora. Two separate pollen types can be distinguished, the type *O. africana* (= *O. chrysophylla*) in which the exine shows a reticulum with large lumina and the type *O. hochstetteri* which has smaller lumina.

In Table 1, specific identifications are given for pollens recognisable by morphological details, which belong to contemporary African genera only known through a single species. These are *Dodonaea viscosa*, *Hagenia abyssinica* and *Ricinus communis*. Within other genera such as *Justicia*, certain species show strong differences. Pollen grains of *Justicia anselliana* are small (~20 µm) and diporate. They have no furrows visible and the exine is of about the

same thickness at the pore and at the equator. *Justicia odora* and *Justicia fischeri* pollen grains are dicolporate with a long visible furrow, their size is $\sim 35 \mu\text{m}$. They cannot be distinguished from each other, and so the fossil has been designated as *Justicia* cf. *odora* type. But this pollen is very different from *J. flava* or *J. striata* which show a very strong thickening of the exine at the equator.

Pollen grains classified under species A are represented by periporate grains of $\sim 20 \mu\text{m}$ with 12 to 15 pores. The pores are well-delimited with an operculum. On the optical sections very fine columellae are barely seen. Such pollen exist among Plantaginaceae (*Plantago*) and Moraceae (*Bosqueia*). Considering the exine structure and the pores, the fossil pollen grains closely resemble those of *Bosqueia phoberos* (= *B. angolensis*) a common tree of the most humid high forest of western Ethiopia and of the rain forest in the Congo. The regular undulations with the verrucate or microechinate sculpturing which characterise the exine of pollen grains in the genus *Plantago* have not been clearly observed on the fossil pollen. In the present East African species of *Plantago*, the pore outline is not well delimited and the pore membrane is covered with granules. No distinct operculum is visible, except in *P. lanceolata*. But in this species, the exine is echinate. The fossil pollen grains cannot be attributed to the genus *Plantago*. They are considered to be close to *Bosqueia*. The possibility that they could belong to an extinct species cannot, however, be excluded.

Pollen grains of species B are tricolporate with granulate colpi membranes, a smooth exine surface and no distinct columella. Similar pollen exists within the 'Meliaceae' family. The fossils, however, are not identical to the living African species of Meliaceae which we have observed.

Pollen grains similar to species A or B have never been recovered from the modern pollen rain content of the East Rudolf or Omo areas. Whatever their precise identification, they have to be included in group A of Table 1.

Comments on the fossil pollen assemblage

Within the fossil pollen assemblage the different taxa have been arranged in five groups (Table 1).

The first group consists of taxa which are not represented today in the vegetation of the East Rudolf area according to the lists established on the basis of the collections of plants made by many authors. This first group has been divided into two parts, A and B. A includes taxa from montane forests of the Ethiopian highlands and B taxa from riverine plant communities. In the second group are

considered taxa from the surrounding semi-desert steppes of the Lake Turkana area. Within a third group are assembled ubiquitous taxa among which grasses are particularly abundant.

As we cannot distinguish the different genera of grasses which belong to distinct communities, we must consider them as a whole. *Typha* is usually found near fresh water but Cyperaceae (sedges), which can be very abundant near saline or alkaline lakes, do exist within savannas among other herbaceous plants. Compositae of the tubuliflorae type as well as Acanthaceae have been classified as ubiquitous taxa because of the imprecise nature of their identification. The fourth and fifth groups are respectively represented by the pteridophyte spores and the unidentified pollen.

The survey of the fossil pollen assemblage (Table 1) shows that among the 47 pollen taxa recorded (pteridophytes included), more than half of them, that is 25 (group 1), are not present in the contemporary East Rudolf vegetation. Nine of them may occur in riverine communities along the Omo river and in the delta, north of Lake Turkana. The other 16 exist, now, in the high forests of south-western Ethiopia, in the Gemu-Gofa and Sidamo provinces, 150 to 200 km east or north of Ileret. The great number of taxa which are not represented in the vegetation of the East Rudolf area today seem to be a remarkable feature of this fossil pollen assemblage.

Comparison with palynological results from modern sediments

Samples of recent sediments were collected as a part of A. K. Behrensmeyer's research into taphonomy. They were taken from lake margin environments at Alia Bay and from modern fluvial ones at the Laga Bura Hasuma and Tulu Bor, both of which are seasonal water courses (Fig. 1).

Table 2 summarises the percentages of pollen of the previously defined groups in these samples, detailed counts will be found in another paper¹⁰. For the palynological data obtained from recent sediments, pollens which belong to taxa from montane and forest communities are considered as allochthonous, that is, transported from the source to the place of deposition. Allochthonous pollen give information on the vegetation of the whole basin.

Implications for regional vegetation patterns

In all the studied samples from recent sediments, the percentages of allochthonous pollen are very small (0.04–2% of

Table 2 Distributions of various groups of pollen taxa in fossil against modern surface sediments of Lake Turkana area, Kenya

	East Rudolf	No.	Highlands		No.	Riverine		No.	Savanna		No.	Ubiquitous		Pteridophytes		Total count
			(a)*	(b)		(a)	(b)		(a)	(b)		(a)	(b)	(a)	(b)	
Fossil	Ileret KFFPI	16	13.6	24.2	9	2.5	4.5	17	20.6	36.6	5	59.6	27.4	2.1	3.8	696
	Alia bay															
	— AB Ia/B ₈	1	0.5	0.9	—	—	—	7	49.9	95.3	4	49.6	3.7	—	—	976
	— AB Ia/B	1	0.3	0.9	—	—	—	4	31.6	91.3	4	68.0	7.5	0.1	0.3	959
	— AB Ia/A	1	0.2	3.0 ⁽¹⁾	—	—	—	2	6.0	86.4 ⁽¹⁾	4	93.8	10.6 ⁽¹⁾	0.	—	957
	— AB Ic/A _{36b}	2	0.5	0.9	—	—	—	7	50.8	97.6	3	48.3	0.8	0.1	0.2	967
Modern	Laga Bura Hasuma transect															
	— BH I/A ₆	1	0.5	1.9 ⁽³⁾	2	4.7	—	26	24.2	94.1	3	28.0	2.7	0.2	0.8	1,000
	— BH I/A ₂	2	0.04	1.2 ⁽²⁾	3	2.5	1.2	24	2.9	86.9	4	94.6	10.6	—	—	4,789
	— BH I/A ₁₂	4	1.5	4.3 ⁽²⁾	4	2.7	2.0 ⁽²⁾	27	29.6	85.8 ⁽²⁾	4	66.2	7.8 ⁽²⁾	—	—	1,000
	Laga Tulu Bor transect															
	— A ₈	3	0.5	0.5	—	—	—	19	85.4	95.3	2	13.6	3.6	0.5	0.5	617
	— A ₄₋₅	3	2.0	3.7	1	0.3	0.6	26	46.5	86.8	4	51.1	8.6	—	—	608
	— A ₁₁	4	1.2	4.8	1	0.1	0.4	18	20.5	81.9	4	77.8	11.6	0.3	1.2	993
	— A ₁₇	2	0.7	2.0	1	0.2	0.5	17	31.7	91.2	4	67.2	5.6	0.2	0.5	558

Modern data are from ref. 11. The localities are shown in Fig. 1.

*% calculations: (a) total count; (b) total count excluding Gramineae and over-represented species (1) Cyperaceae, (2) *Combretum*, (3) *Combretum* + *Acalypha*.

Table 3 Comparative pollen taxonomic diversity (number of taxa) in Plio-Pleistocene against modern surface sediments from Omo and East Rudolf

Age	Samples	Highlands	Riverine	Savanna	Ubiquitous	Total
Modern	M AB	1.2	0	5	3.7	18
Fossil	M BH+TB	2.7	1.7	22.4	3.6	68
1.6-1.5 Myr	KFFP1	16	9	16	5	46
2.2-2.15 Myr	Omo E-4	5	1	10	3	19
2.45-2.4 Myr	Omo C-9	11	6	18	4	39
2.45-2.4 Myr	Omo C-7/C-8	10	6	23	3	42

M = mean of results from Table 2. C-8, C-9, E-4 are stratigraphic units from the Shungura Formation, Lower Omo Valley, Ethiopia.

the total count, or 0.5-4.8% of the total from which grasses are excluded). There are slight differences between the percentages of lacustrine and fluvial allochthonous taxa observed in the various samples. (Table 2).

By contrast the number of riverine and highland taxa is much more important in the fossil assemblage than in any recent samples (Table 3). Altogether the percentages of those taxa represent 16.2% of the total pollen (Table 1) or 28.7% of the total pollen after Gramineae are excluded (Table 4). Such large percentages of highland taxa have been recorded in a pollen spectrum of recent Omo river deposits only from a site 50 km North of the Omo delta and 150 km north of Ileret (Table 4). In this recent assemblage, allochthonous pollen from the Ethiopian highlands is brought down streams.

Considering the fossil data, there seem to be three principle lines of explanation for the higher percentages of allochthonous pollen compared with recent spectra: first, the allochthonous pollen may formerly have been introduced from the north-east by a large river which has now ceased to flow; second, the incidence of allochthonous pollen in recent sediments may have been artificially depressed by recent deforestation caused by human interference, and third, at the time of the deposition of the fossil sample, different environmental conditions may have permitted the growth of montane forest species closer to Ileret than today.

The first seems unlikely through a detailed reconstruction of the palaeogeography¹¹. Also bones and hominid remains, particularly abundant in area 12, have not been subjected to noticeable reworking. Modern sediments from the delta margin transect of Alia Bay are considered by Behrens-meyer as the closest model for deposition of the Lower Pleistocene sediments at Ileret. On the basis of the geological data, stream transport of pollen seems an unlikely explanation of the high value of mountain taxa in the fossil pollen assemblage. Furthermore, if the fossil pollen assemblage at East Rudolf was from a fluvial environment, it should also show a higher concentration of riverine taxa than are in fact recorded (compare data for recent sediments of the Omo River, Table 4). No pollen of *Zyzygium*, *Celtis* or *Ziziphus*, all common trees of riverine plant communities, have been recorded in this sample. Even if a part of the allochthonous fossil pollen was introduced by fluvial transport, it should be noted that the modern rivers on the eastern side of Lake Turkana do not transport comparable pollens. Also, the

whole picture given by the pollen spectrum is consistent with deposition in a situation between the distributary channels of a delta.

For the second possible explanation, it is apparent that if the differences between recent and fossil assemblages are to be attributed to intensified human activity, then all early fossil assemblages from Omo and East Rudolf should differ from the modern ones in a similar manner. Tables 3 and 4 demonstrate the contrary. Strong fluctuations in the amounts of montane forest pollen have already been noticed in Plio-Pleistocene sediments of the Shungura Formation in the lower Omo Valley¹².

Explanations of the contrast between the fossil and recent spectra which depend purely on differences of environment of deposition seem highly unlikely in view of the recent demonstration that these do not greatly influence the relative quantity of allochthonous pollen¹⁰. I therefore favour the third explanation, namely that the fossil sample reflects climatic and ecological environmental conditions differing significantly from those of the present day. At Ileret, 1.5⁶ Myr ago, climatic conditions must have been cooler and more humid than today, and more favourable to extensive forests.

Implications for local vegetation patterns

In the fossil pollen assemblage from Ileret (Table 1) group 2, which contains the taxa recorded today in the East Rudolf vegetation, shows a great abundance of *Acacia*+*Capparaceae*+*Commiphora* and *Salvadoraceae*, all common trees of the dry woodland. Among the herbaceous taxa, *Chenopodiaceae*+*Amaranthaceae* are the most abundant, with the *Euphorbiaceae*. No interpretation can be made of the *Euphorbia* pollen since ~ 100 species with different ecologies are found in East Africa. It is not the *Euphorbia grandicornis* commonly found in this subdesert area.

Chenopodiaceae and Amaranthaceae

Among the *Chenopodiaceae* it was not possible to increase the precision of pollen identification to the generic level. The fossil pollens are very similar to pollens of *Suaeda* or *Fadenia*, very abundant today in the vegetation surrounding Lake Turkana. Altogether *Chenopodiaceae* + *Gramineae* + *Amaranthaceae* + *Cyperaceae* represent ~ 60% of the fossil pollen count. The recent lacustrine samples from East Rudolf are extremely consistent with high values

Table 4 Comparative pollen composition (as percentages) of Plio-Pleistocene deposits against modern surface sediments from Omo and East Rudolf

		Highlands	Riverine	Savanna	Ubiquitous	Pteridophyte	Total pollen count
Modern	Omo River	29.5	20.3	14.2	24.7	5.8	1,000
	M AB	1.4	—	92.6	5.6	0.1	3,859
	M BH+TB	2.6	0.7	88.8	7.2	0.4	9,565
Fossil	KFFP 1	24.2	4.5	36.6	27.4	3.8	696
	Omo E-4	6.4	0.3	84.4	5.7	1.0	1,062
	Omo C-9	23.6	11.8	12.8	49.6	0.7	693
	Omo C-7/C-8	38.8	6.0	23.0	24.9	1.0	1,072

% expressed as percentages of total count excluding Gramineae and over-represented species.

of Chenopodiaceae associated with Cyperaceae and Gramineae (97–99%) while the fluviatile series are more variable (62–96%).

Pollen analyses of surface sediment samples have shown high percentages of Chenopodiaceae in the subdesert and desert areas of the Sahara^{13,14}, in the arid zones of the Middle East, in Iran¹⁵, and also in the southwestern USA (such as Arizona¹⁶) as well as in dry continental areas of the USSR¹⁷. Chenopodiaceae are numerous in places with high salinity or alkaline soils.

On the basis of modern pollen rain studies, authors agree that high values of Chenopodiaceae pollen are obtained near places where the plants are found. Their density decreases rapidly with distance from the source. For this reason, high Chenopodiaceae frequencies in fossil data are basically the result of local conditions rather than regional ones. In this particular fossil assemblage, related to a delta margin environment, a percentage of Chenopodiaceae much lower than in the recent Alia Bay samples may indicate a lake less alkaline than the present one.

Woodland and bushland

Percentage calculations of the various savanna taxa included in the groups 2+3 (Table 1) of the fossil pollen assemblage are given in Table 5. They provide information on the composition of the local vegetation around Lake Turkana in the past. It should be noticed that the pollen composition of the fossil assemblage differs from all the studied modern spectra in containing a great quantity of *Acacia* and *Commiphora*. The ratio between arborescent and non-arborescent taxa calculated from Table 5 has about the same values as for recent pollen spectra from surface samples collected beneath tree cover near the Tulu Bor River, east of Lake Turkana¹⁰. We consider that the vegetation near the lake at the time of deposition of the sediment, was more wooded than today, and can be regarded as *Acacia-Commiphora* bushland. The great percentage of pollen of Salvadoraceae, in which the identification of *Salvadora* from *Dobera* is not possible, could reflect the mesic environment near a river delta.

Discussion

Because of the present lack of data on the pollen rain in tropical lowlands, great caution is needed in the palaeoclimatic interpretation of this pollen analysis. A pollen assemblage does not exactly reproduce the detailed composition of the regional vegetation, but it reflects the essential character of the flora which existed at the time of deposition. Such information on vegetation would not be available without palynological studies.

The composition of the plant community and the resultant pollen rain can be related by correlation factors to allow for the differential pollen production by wind-pollinated and insect-pollinated plants. For the moment no great precision is possible. But we have, in effect, taken those factors into account by comparing the fossil assemblage with assemblages of recent pollen deposited in the same area. More sophisticated treatment must await the compilation of much more comparative data on pollen production, absolute and relative dispersal in such tropical areas. In spite of the reservations just expressed, it remains true that plants constitute the most sensitive indicators of climatic, hydrological and pedological conditions of their habitat. The ecological requirements for certain species and vegetation communities are so precise that they can be used to characterise particular climatic regimes.

The fossil pollen assemblage from Illet seems to permit a reasonably definite characterisation of the flora in the area, 1.5 Myr ago. The prominence of montane forest elements is particularly striking. Since it appears that this feature cannot be explained entirely by long distance wind or water transport, it seems clear that vegetation of highland forest type must have existed closer to the basin

Table 5 Distribution of savanna taxa (groups 2+3 of table 1) in the fossil pollen spectrum KFFP1

	No.	%*	%†
Trees and Shrubs			
<i>Acacia</i>	18	3.4	13.8
<i>Capparaceae</i> + <i>Grewia</i> + <i>Commiphora</i> + <i>Cordia</i>	12	2.3	9.2
<i>Salvadora</i>	37	7.0	28.5
Others	3	0.6	2.3
Herbaceous			
<i>Acanthaceae</i>	3	0.6	2.3
<i>Euphorbiaceae</i>	35	6.7	26.9
<i>Amaranthaceae</i>	3	0.6	2.3
<i>Compositae</i>	14	2.7	10.8
Others	5	0.9	3.8
Total	130		
Gramineae	303	57.7	
Cyperaceae + <i>Typha</i>	92	17.5	
Total	525		

* % Calculations with Chenopodiaceae excluded. Pollen sum 525.

† With Chenopodiaceae + Cyperaceae + *Typha* Gramineae excluded. Pollen sum 130.

margins. Further, because the contrast between recent pollen assemblages and the fossil one cannot have arisen simply from recent deforestation, the climate must surely have been somewhat cooler or wetter at the time of deposition of the sample. The vegetation in the vicinity of the sample site was dominated by Gramineae and Chenopodiaceae appropriate to the margins of a slightly saline or alkaline lake. On the other hand, the unusually good representation of *Acacia*, *Commiphora* and *Salvadora* implies a shrub and tree cover that was more dense than that prevalent in the basin today. Altogether the indications are of a climate that was neither excessively humid nor semi-arid.

The sample was taken from strata which contain early hominid fossils and archaeological remains of the Upper Member of the Koobi Fora Formation. The pollen spectrum of the Koobi Fora documents conditions existing at some point during the occupation of the area by evolving hominid populations. The palaeoenvironmental evidence provided by this sample, however, refers to a relatively short timespan during the deposition of the sediment containing the pollen spectrum. To assess the extent to which the pattern observed was stable, fluctuating or subject to persistent long term trends, we will need to procure spectra from other layers.

I thank Glynn Isaac and Richard Leakey for help, and the CNRS and the NSF for support. A. K. Behrensmeyer, G. Isaac and I. Findlater helped with the field work, with stratigraphic interpretation and in the preparation of the report. J. B. Gillett and C. Kabuye provided botanical specimens from the East African Herbarium, Nairobi. C. Vanbesien and G. Buchet gave assistance in the laboratory.

Received May 3; accepted October 9, 1976.

1. *Earliest Man and Environments in the Lake Rudolf Basin: Stratigraphy, Paleocology and Evolution* (edit. by Coppens, Y., Howell, F. C., Isaac, G. L., and Leakey, R. E.), (University of Chicago Press, 1975).
2. Leakey, R. E., *Am. J. phys. Anthropol.*, **41**, 245–250 (1974).
3. Isaac, G. L., Leakey, R. E., and Behrensmeyer, A. K., *Science*, **173**, 1129–1134 (1971).
4. Bowen, B. E., and Vondra, C. F., *Nature*, **242**, 391–393 (1973).
5. Findlater, I. C., in *Earliest Man and Environments in the Lake Rudolf Basin: Stratigraphy, Paleocology and Evolution* (edit. by Coppens, Y., Howell, F. C., Isaac, G. L., and Leakey, R. E.), (University of Chicago Press, 1975).
6. Behrensmeyer, A. K., thesis, Harvard University (1973).
7. Fitch, F. J., Findlater, I. C., Watkins, R. T., and Miller, J. A., *Nature*, **251**, 213–215 (1974).
8. Curtis, G. H., Drake, R. E., Cerling, T. E., Cerling, B. L., and Hampel, J. H., *Nature*, **258**, 395–398 (1975).
9. Riollot, G., and Bonnefille, R., *Pollen et Spores*, **XVIII**, no. 1, 67–92 (1976).
10. Findlater, I. C., thesis, Univ. London (1976).
11. Bonnefille, R., and Vinceus A., *Proc. Tenth INQUA Cong., Birmingham, 1977* (in the press).
12. Bonnefille, R., in *Earliest Man and Environments in the Lake Rudolf Basin: Stratigraphy, Paleocology and Evolution* (edit. by Coppens, Y., Howell, F. C., Isaac, G. L., and Leakey, R. E.), (University of Chicago Press, 1975).
13. Van Campo, M., in *Northeast African and Levantine Pleistocene Prehistory* (edit. by Wendorf, F.), (Dallas, 1975).
14. Cour, P., et al., in *3rd Int. Palyn. Conf. Novosibirsk, USSR* (1971).
15. Wright, H. E., Mcandrews, J. J., and Van Zeist, W., *J. Ecol.*, **55**, 415–443 (1967).
16. Potter, L. D., Schoenwetter, J., and Oldfield, F., in *Late Pleistocene of the Southern High Plains* (edit. by Wendorf, F., and Hester, J.), (Fort Burgwin Research Center, 9, 1975).
17. Pokrovskaja, I. M., *Bull. B.R.G.M.—C.N.R.S.*, **26**, 434 pp (1950).

Wind shear observations in thunderstorm density currents

F. F. Hall, Jr & W. D. Neff

Wave Propagation Laboratory, National Oceanic & Atmospheric Administration, Environmental Research Laboratories, Boulder, Colorado 80302

T. V. Frazier

Department of Physics, University of Nevada, Reno, Nevada 89507

Acoustic sounder observations of thunderstorm density currents reveal a complicated but ordered internal structure. Fast-response anemometers on a 150-m tower reveal a succession of internal shear layers which occur following the leading portion or nose of the current. The measured wind shear revealed by the anemometers is found to be a function of the temperature differential between the ambient air and the interior of the gust, measured near the surface. An heuristic model is developed to explain this observation.

DENSITY currents flowing out from thunderstorms, sometimes referred to as gust fronts or microfronts, can constitute a hazard to the landing and takeoff of aircraft. The associated wind shear results in the aircraft experiencing rapid changes in air speed; vertical shears $> 0.1 \text{ s}^{-1}$ in the lowest 100 m have been shown to be hazardous to large, swept-wing, jet-powered aircraft in a study by Snyder¹. An improved knowledge of density current dynamics and structure is also important in understanding the interaction of the thunderstorm with its environment. Since the cold air outflows from storms are statically stable, but highly turbulent, such currents exhibit large acoustic index of refraction fluctuations, leading to strong acoustic echoes when the flows are probed with sound waves. Using an acoustic sounder as a guide in the interpretation of meteorological tower data we present new observations of the internal structure and shear in gust fronts, and find a strong correlation between the strength of the current and its temperature contrast with the ambient air.

Wind shear measurements

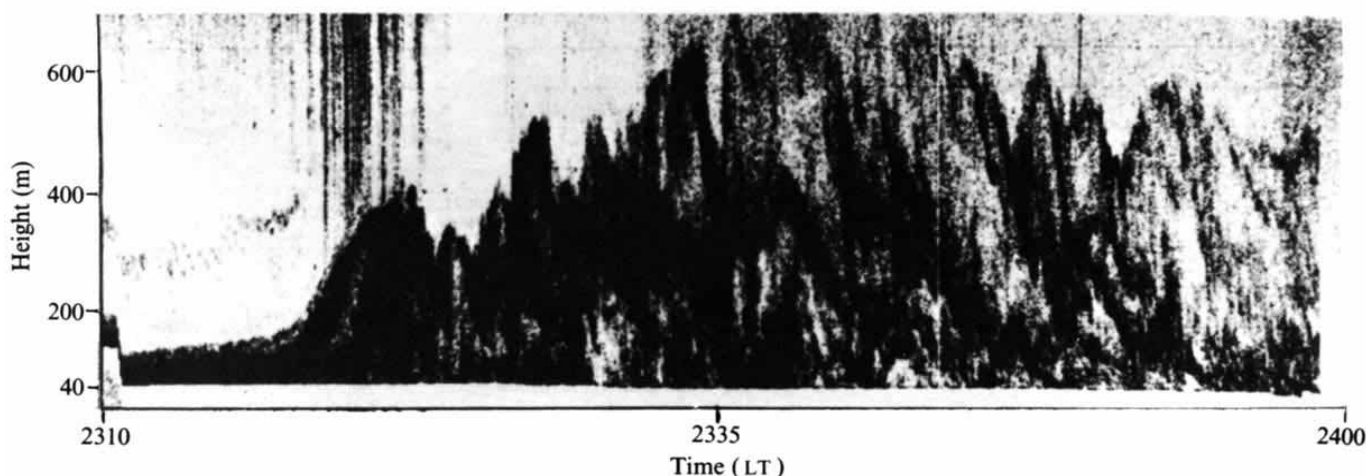
During field experiments in 1972 and 1974, five different thunderstorm density currents were observed at the National

Oceanic and Atmospheric Administration (NOAA), Haswell, Colorado site. The meteorological tower at Haswell, 150 m tall and located 300 m from an acoustic sounder, was instrumented at 30-m intervals with fast response wind and temperature sensors; data were recorded at 1.0-s intervals. In addition, heavy-duty wind sensors were located at 15 m and 150 m, and were able to withstand the high winds which in two cases destroyed the fast response instruments.

In the two more severe storms observed, wind-generated noise at the acoustic antenna masked the reception of echoes, but for the other cases, the internal shear structure was observed in detail with the sounder. The structure for the August 11, 1972 current is shown in Fig. 1. This outflow originated near a line of small thunderstorms which had levelled off at a peak altitude of 8.5 km above sea level, and were observed to dissipate 20 km NE of Haswell at 2300 by the National Weather Service weather radar in Limon, Colorado. When last observed the storms were moving 6 m s^{-1} from an azimuth of 330° . The maximum wind speeds within the current were 5 m s^{-1} from between 280° and 340° . This density current flow was opposed to an ambient flow of 10 m s^{-1} from 135° before the event, producing a vector change in wind speed, Δu of 15 m s^{-1} . A temperature drop of 2°C at 30 m occurred with the passage of the microfront or head of the current. Maximum shears at the lower edge and in the interior of the current reached values of $\partial u / \partial z = 0.13 \text{ s}^{-1}$ as measured by the tower bivanies; the strongest shear events correlated with temperature shifts measured on the tower and with the observation of scattering regions on the acoustic sounder facsimile record. This correlation is to be expected since theory predicts that the acoustic scattering is produced by small scale temperature fluctuations associated with turbulence in regions of temperature and wind gradients.

This prediction has been verified quantitatively by Neff² for a number of cases including elevated scattering layers. The

Fig. 1 Acoustic sounder facsimile record obtained on August 11, 1972 at Haswell, Colorado, of a density current originating from a line of weak storms more than 20 km away. Wind noise associated with the passage of the current nose led to the vertical noise-lines in the upper part of the record at 2320. The postulated shears and temperature gradients at the edge of the current, and in descending layers within the current, are revealed as darker regions on the facsimile record because of increased acoustic scattering cross sections.



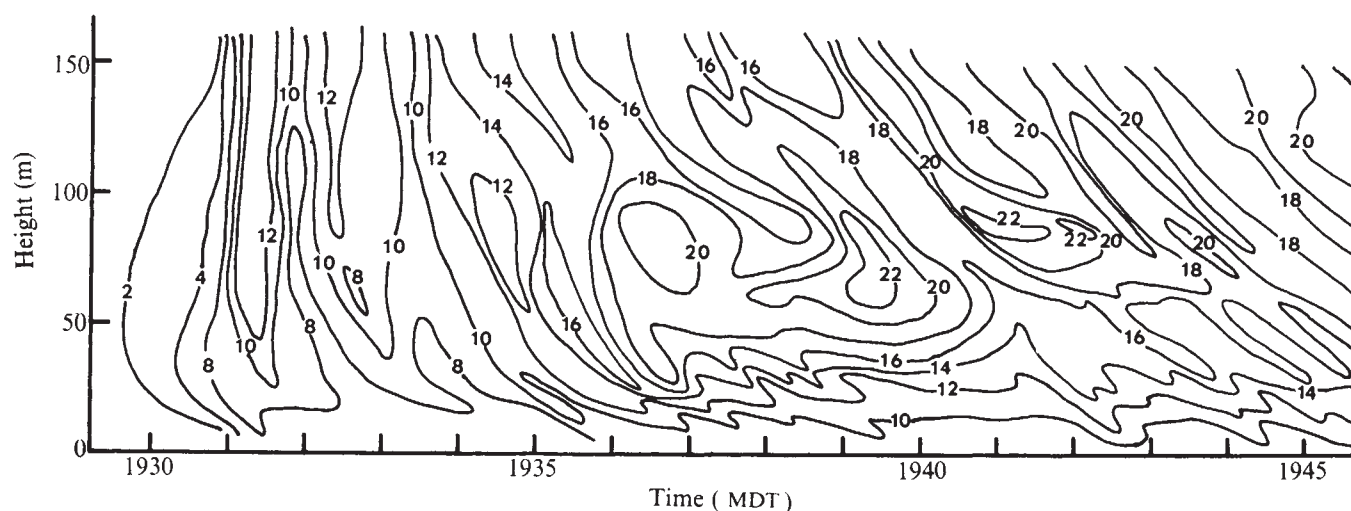


Fig. 2 Isotachs in m s^{-1} drawn against height and time as derived from wind measurements at six levels on the Haswell tower, 10-s averaged data for the density current of August 5, 1972. The resulting pattern of shear is consistent with the layered structure shown by acoustic sounder observations of much weaker currents as in Fig. 1.

thin, tilted echo layers, internal to the current but above the height of the tower, are thus presumably of such an origin. Although not verifiable from the present data, the local enhancement of the shear and consequent production of turbulence may arise from internal waves generated at the interface or large-scale overturning within the current.

Two more vigorous density currents from closer and larger thunderstorms passed the tower on August 2 and 5, 1972. In these cases, the wind noise was so great that acoustic records could not be obtained. By using the August 11 facsimile record as a guide in interpreting the wind data from the tower, however, a consistent set of isotachs could be plotted for the August 5 event following the form suggested by Fig. 1, with tilted shear layers gradually sloping or descending with time. These are shown in Fig. 2, plotted from data averaged over 10 s. One minute of data averaging proved to be too long to delineate the thin shear regions within the currents; therefore, internal shear layers were not revealed in the recent comprehensive thunderstorm outflow study by Goff⁴, who used 2-min wind averages.

From Fig. 2, it is immediately obvious that the vertical shear $\partial u / \partial z$ in the lower 50 m is consistently large and above the aircraft safety criterion set by Snyder¹. It must be realised that the spatial slope of the higher shear zones is greatly accentuated in Figs 1 and 2; if one assumes the zones are advected with the mean wind, the actual slopes for the shear

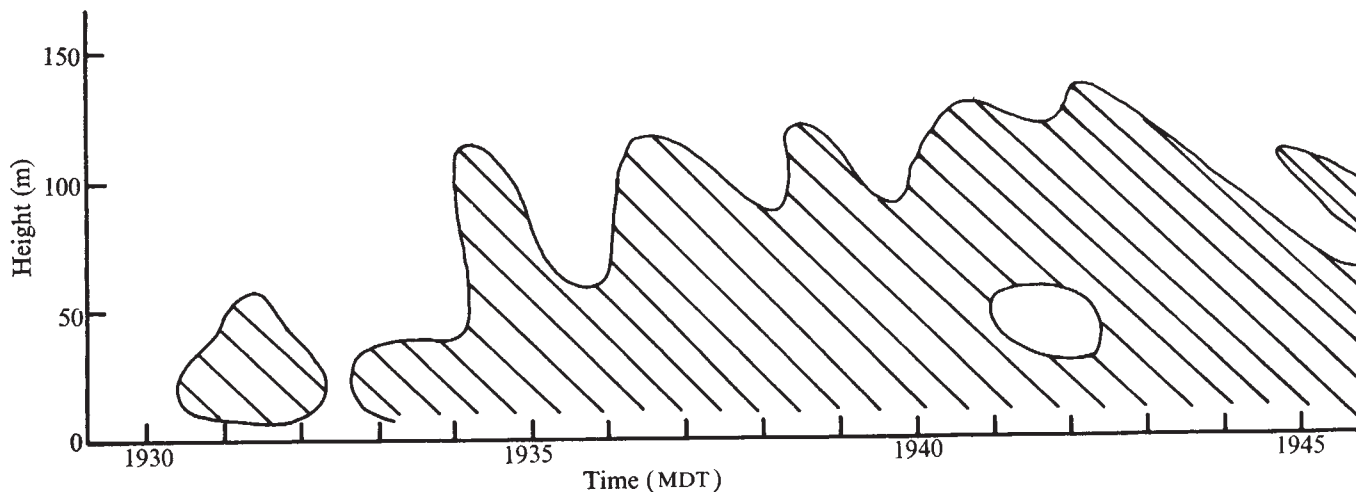
regions at 100 m are found to be $< 2^\circ$. The fluctuating nature of the shear at higher levels yields an undulatory pattern of shears $> 0.1 \text{ s}^{-1}$. Regions where this critical shear value is exceeded are shown in Fig. 3, indicating that even 16 km from this quite averagely sized prairie thunderstorm (with radar echoes extending to an altitude of 14.6 km), the clear air wind shear exceeded the safety limits consistently in the lower 100 m for > 10 min. Shortly after 1946 on August 5, a gust of more than 25 m s^{-1} destroyed two of the light weight propellers on the fast response sensors, or bivanes, eliminating further data.

The even more energetic density current of August 2, 1972 arose from a storm just 5 km away with radar tops observed to be above 17 km. Maximum velocities in this current exceeded 42 m s^{-1} , which quickly destroyed all bivanes on the tower. A recorded wind shear of 0.26 s^{-1} was observed over the 135-m separation of the two more rugged but slower response wind sensors. Thus the actual shear internal to the current (which otherwise might have been measured with 30-m height resolution provided by the bivanes) was probably greater than that recorded over the greater height increment separating the rugged sensors.

A model of density current wind shear

A feature common to the density current events observed was the drop in temperature with the arrival of the first gust. Empirically, it was found that the larger the temperature drop,

Fig. 3 Height against time plot of those regions where the wind shear exceeded 0.1 s^{-1} for the August 5, 1972 density current at Haswell, Colorado.



the greater the observed shear, consistent with the primitive equation model of gust fronts by Mitchell⁵. This suggested that temperature measurements relatively near the surface might serve as a quantitative predictor of the severity of density current shears. We now outline a simple model relating temperature drop to wind shear.

An obviously relevant parameter in density current modelling is the speed of the current, u_g . This is given by Turner⁶ as

$$u_g = \alpha [gH(T_a - T_g)/T_a]^{1/2} \quad (1)$$

where g is the acceleration of gravity, H is the depth of the current following the head, T_a the ambient air temperature, and T_g the temperature of the gust. Equation (1) comes from hydraulic theory. When dissipative processes are neglected the constant, α , is $\sqrt{2}$. Laboratory investigations yield an empirical value for α of 1.1 (ref. 3).

We consider first the shear along the upper boundary of the current near its nose. In this region of warm air overlying the colder current, we hypothesise that the turbulence generated there self-adjusts to gradient Richardson numbers, Ri (~ 0.2), as observed in thin inversion surfaces in the real atmosphere (Turner⁶, Hall *et al.*⁷). From the definition of Ri we write

$$\frac{\partial u}{\partial z} = \frac{1}{Ri} \frac{g}{\theta} \frac{\partial \theta}{\partial z} \bigg/ \frac{\partial u}{\partial z} \quad (2)$$

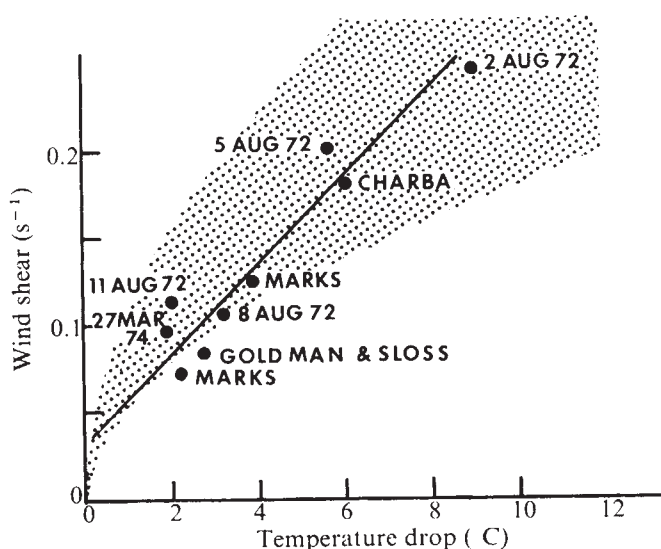
This can be approximated as

$$\frac{\partial u}{\partial z} \approx \frac{1}{Ri} \frac{g}{T} \frac{\Delta T}{\Delta u} \quad (3)$$

where the vertical gradients are assumed to occur over the same depths, the temperature lapse rates within and outside of the current are nearly equal, and $\Delta T = T_a - T_g$, measured near the surface. Assuming the ambient flow velocity is zero, $\Delta u = u_g$. Then equation (3) becomes

$$\frac{\partial u}{\partial z} = \frac{1}{\alpha Ri} \left(\frac{g \Delta T}{HT} \right)^{1/2} \quad (4)$$

Fig. 4 Measured wind shear plotted against temperature drop from the ambient atmosphere to the density current. The experimental data points, as described in the text, nearly fit a straight line, while the overlay stippled area represents approximate bounds on the shear predicted by equation (7).



Taking $H = 500$ m, which the sounder reveals to be a characteristic value for a number of such currents observed, $Ri = 0.2$, $\alpha = 1.1$ and $T = 300$ K, we have

$$\frac{\partial u}{\partial z} = 0.037 (\Delta T)^{1/2} \quad (5)$$

For $\Delta T > 7^\circ\text{C}$ at the upper boundary of the current, equation (5) predicts wind shears > 0.1 , the safety limit derived by Snyder¹.

At the lower boundary of the current, where the shear is actually measurable on the tower, the speed must go to zero through some boundary layer thickness δ . We can then use equation (1) to estimate

$$\frac{\partial u}{\partial z} = \frac{u_g}{\delta} = \alpha \left(\frac{gH\Delta T}{\delta^2 T} \right)^{1/2} \quad (6)$$

Taking again $H \approx 500$ m and $50 \text{ m} < \delta < 100$ m (typically observed values) we have

$$0.044 (\Delta T)^{1/2} < \frac{\partial u}{\partial z} < 0.089 (\Delta T)^{1/2} \quad (7)$$

expressions surprisingly similar to equation (5), but predicting shears near the surface somewhat larger than at the upper boundary of the current.

A summary of the maximum observed wind shear for five density currents as recorded at Haswell is shown in Fig. 4, where the dated data points show the values recorded by bivanos on the 150-m tower, except for the August 2, 1972 event. Other points shown were recorded by Charba⁸ and by Marks⁹ at the WKY tower near Oklahoma City. The Goldman and Sloss¹⁰ data were obtained at the 150-m Kennedy Space Center, Florida tower. A least-squares linear fit to the data, relating wind shear to temperature drop observed in the density current, is shown as a solid line. The equation for this line is

$$\frac{\partial u}{\partial z} = 0.024 \Delta T + 0.03 \quad (8)$$

The area bounded by the limits of equation (7) is plotted as the stippled overlay which agrees well with the data. Equation (5) would plot near the lower bound of the stippled zone. The non-zero intercept in Fig. 4 probably reflects our neglect of the background shear.

The fact that the observed shear varies nearly linearly with ΔT may reflect an indirect dependence of H on ΔT as suggested in the two-dimensional primitive equation model of Mitchell⁵. Our simple heuristic model also avoids the variability in the static stability of the ambient air. Equation (7) is thus useful mainly to set approximate bounds on the shear using values of H typical of the few cases observed by the sounder.

If the simple relationships shown in Fig. 4 prove to be typical for an even larger data set, it would be a relatively simple matter to measure ΔT on a grid surrounding airports to warn of the wind shear produced by density currents moving across the area.

Conclusions

We conclude that even at distances > 16 km from thunderstorms, the wind shear in density currents from such storms can be a hazard to aircraft landing and taking off. Unless such density currents are delineated by blown dust, as in desert regions, they will be undetectable to the unaided observer. The temperature drop associated with these currents, however, gives a good measure of the wind shear to be expected. In addition, the ability of the acoustic sounder to detect such turbulent shear layers may provide new insight into the internal dynamics and structure of such currents in the atmosphere.

We thank D. W. Beran, W. H. Hooke, C. G. Little and P. A. Mandics for discussions. G. Gertig assisted with the data reduction and the plotting. Support for this study was provided by the Federal Aviation Administration.

Received May 24; accepted October 14, 1976.

- ¹ Snyder, C. T., *Analogue study of longitudinal response to wind shear and sustained gusts during landing approach* (NASA TN D-4447, Ames Research Center, Moffett Field, California, 1968).
- ² Neff, W. D., *Quantitative evaluation of acoustic echoes from the planetary boundary layer* (NOAA Tech. Rep. ERL 322-WPL 38, Environmental Research Laboratories, Boulder, Colorado 1975).
- ³ Benjamin, T. B., *J. Fluid Mech.*, **31**, 209–248 (1968).

- ⁴ Goff, R. C., *Thunderstorm-outflow kinematics and dynamics* (NOAA Tech. Memo. ERL NSSL-75, National Severe Storms Laboratory, Norman, Oklahoma, 1975).
- ⁵ Mitchell, K. E., *A numerical investigation of severe thunderstorm gust fronts* (NASA Report CR-2635, Pennsylvania State Universities, Contr. NAS8-27334, 1975).
- ⁶ Turner, J. S., *Buoyancy Effects in Fluids* (Cambridge, University Press, 1973).
- ⁷ Hall, F. F., Jr, Edinger, J. G., and Neff, W. D., *J. appl. Meteorol.*, **14**, 513–523 (1975).
- ⁸ Charba, J., *Gravity current model applied to analysis of squall-line gust front* (NOAA Tech. Memo. ERL NSSL-61, National Severe Storms Laboratory, Norman, Oklahoma, 1972).
- ⁹ Marks, J. R., *Acoustic radar investigations of boundary layer phenomena* (Sci. Rep. on NAS8-28659, Department of Meteorology, University of Oklahoma, Norman, Oklahoma, 1973).
- ¹⁰ Goldman, J. L., and Sloss, P. W., *Structure of the leading edge of thunderstorm cold-air outflow, 75–79* (Sixth Conference on Severe Local Storms, American Meteorological Society, Boston, 1969).

Cell surface distribution of lectin receptors determined by resonance energy transfer

S. M. Fernandez & R. D. Berlin

Department of Physiology, University of Connecticut Health Center, Farmington, Connecticut 06032

The surface topography of concanavalin A (con A) bound to normal and transformed murine fibroblasts has been studied by a new technique involving fluorescence resonance energy transfer (RET). RET can provide a high resolution "map" of the distances separating con A-receptor complexes in single living cells. The distribution of con A is non-random in both normal and transformed cells, but sites are more closely approximated in the transformed. Approximation is induced by the con A but occurs at extremely slow rates indicating that the topography is not primarily determined by simple diffusion of complexes.

THE enhanced agglutinability of tumour compared with normal cells by plant lectins such as concanavalin A (con A) has led to the extensive use of lectins as "probes" of surface organisation. Yet the essential description of the binding of lectins to cell surfaces is controversial and incomplete. Although the number of binding sites per cell is equal^{1,2}, the surface density of bound con A is probably greater on transformed than normal cultured fibroblasts³. The distribution of con A over the surface may also be markedly different. The aggregation of con A-receptor complexes (C-R complexes) into clusters has been considered characteristic of the malignant cell⁴. The formation of these clusters depends on the movement of C-R complexes. When cells are prefixed with aldehydes and then labelled, the distribution of C-R complexes appears disperse or "random" on transformed as well as normal fibroblasts⁵. Thus C-R complexes are said to be more "mobile" on transformed cells. Similarly, after colchicine treatment, bound con A is distributed in large aggregates or caps on transformed but not on normal cultured fibroblasts⁶. These differences have been attributed alternatively to the intrinsic fluidity of the membrane or to submembranous structures, such as microtubules, which can modify the topography and apparent mobility of C-R complexes.

In our view, the available methodology has had two major limitations. First, the degree of topographical order has been difficult to quantify from electron micrographs. The terms "random", "dispersed" or "clustered" used to characterise the surface distribution of C-R complexes are not defined. Only marked degrees of clustering are obvious and subtle changes in topography cannot be determined. The assessment of order is further complicated by problems of statistical sampling of cells or regions of their surfaces.

Second, the distribution of C-R complexes on living cells has been studied only by fluorescence microscopy. This technique

permits direct observation of gross changes in C-R distribution ("random", large aggregates or caps) but does not allow observations at high resolution in dynamic conditions. Thus, the history of a given topographical distribution as seen on surface replicas by electron microscopy can only be reconstructed indirectly.

We describe here an approach to the analysis of surface topography that obviates some of these problems. We have utilised fluorescence resonance energy transfer (RET) between molecules of surface-bound con A to measure the distances separating them. The method makes possible the development of a high resolution "map" of the topography of C-R complexes. We have analysed the topography of C-R complexes on normal and transformed fibroblasts. The results provide a quantitative description of differences in the topographical organisation of C-R complexes between normal and transformed cells. The time course over which some of these differences evolve is very long and seems to exclude the rate of receptor diffusion as a major determinant of topographical organisation.

High resolution topographical mapping by RET

RET occurs when a donor chromophore which is excited by the absorption of light transmits some of the absorbed energy to another (acceptor) chromophore some distance away. This transfer does not involve the actual reabsorption of light by the acceptor but requires that the distance between the chromophores be relatively close (usually not exceeding 100 Å). Energy transfer varies most importantly as the inverse of the sixth power of the distance separating the chromophores. From the extent of transfer, quantitative theory allows an estimate of the distance between chromophores⁷. By labelling subpopulations of microtubule subunits or membranes with different chromophores, we have used the technique for the study of microtubule polymerisation and membrane-microtubule interaction *in vitro*⁸.

In the study reported here the con A used for cell labelling was a mixture of two populations separately conjugated to donor or acceptor fluorochromes. The ratio of acceptor to donor and the absolute dye to protein ratios were chosen such that at the surface density of bound con A an approximation of C-R complexes would be observed as an increase in RET.

Our system has two characteristics not frequently encountered in recent applications of RET. First, in our system the distance between donor and acceptor is not fixed. The chromophores are not placed at different sites on the same molecule as they are in applications in which RET can be used as a "spectroscopic ruler"⁹. Instead, because the chromophores are placed on different molecules statistically distributed over the two-

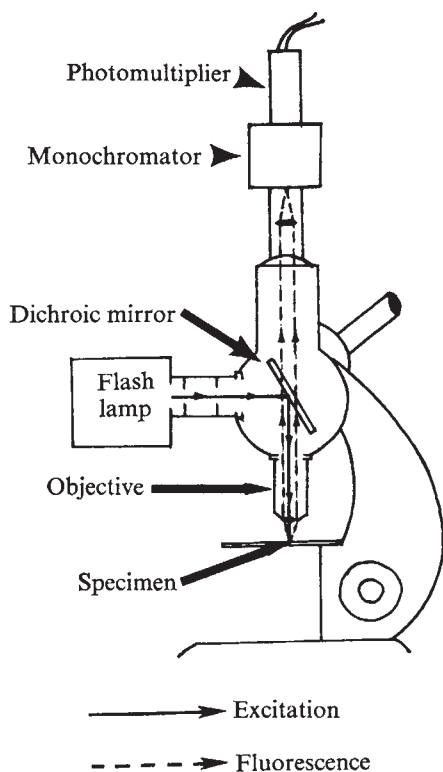


Fig. 1 Modified Zeiss microscope equipped for epiillumination. The incident light source was a Photophysics nanosecond flash lamp. The source was focused through a UGI filter on to a Zeiss No. 450 dichroic mirror. Light was reflected downward and focussed on to the sample through the microscope objective (usually a planachromat 1.25 NA $\times$ 100). Specimen fluorescence was collected in the objective, passed through the mirror, focussed on a grating monochromator and thence to a RCA 8850 photomultiplier. A monophoton counting device was used to record the light incident on the photomultiplier. A detailed description of the apparatus will be given separately (S.M.F., in preparation).

dimensional cell surface, the distance between chromophores varies in a corresponding statistical manner. An increase in RET thus corresponds to a statistical packing or aggregation of donors and acceptors. The analysis of this situation is not novel. RET between mixtures of chromophores in solution was the first system to be analysed by Förster⁷ and other physical chemists (for example, ref. 10). Second, in our analysis only changes in RET are considered useful. It has been emphasised repeatedly that the precise calculation of distances between donors and acceptors based on RET depends on the orientation and mobility of absorption and emission dipoles¹¹. This information is rarely available. We assume only that the orientation factors remain essentially constant throughout our experiment and focus attention on temporal variations of RET. Changes in RET represent changes in the distance separating donors and acceptors. We emphasise, however, that these relative distances correspond to changes of only a few Ångströms, for no energy transfer is detectable at separations exceeding 100 Å.

Observations were made with a modified fluorescence microscope using reflected light (epiillumination). This method of illumination provided sufficient intensity at the specimen when weak light sources (such as a flash lamp) were used. Fluorescence was detected using a sensitive monophoton counting device. The apparatus is shown in Fig. 1.

The ratio of acceptor to donor chromophores in mixtures used to label cells has proved critical. A model system in which this ratio is varied experimentally and related to the RET illustrates the principle and reveals several other features of our rationale. For these experiments, we used tubulin (tubulin and con A are approximately equal in molecular weight) which was

available in bulk and could be labelled conveniently to various ratios of dye to protein. We prepared mixtures of tubulin separately labelled with dimethylamino-naphthalene sulphonylchloride (DANS) or rhodamine isothiocyanate (RITC), and added various amounts of unlabelled tubulin. The proteins were cross linked with glutaraldehyde, concentrated and smeared on to glass slides for examination of fluorescence in our instrument. The precise calculation of RET from donor and acceptor fluorescence is a useful method for analysis of solutions and has been detailed elsewhere¹². Information on absorption by donor and acceptor and on acceptor quantum yield, however, is required and can be obtained only with great difficulty through the microscope. Since RET results in an increase in acceptor emission (sensitised fluorescence) and a decrease in donor fluorescence, we took the ratio of acceptor to donor fluorescence as an approximate measure of RET. This measure proved useful and, we feel, appropriate in view of our emphasis on relative changes and the uncertainties in obtaining less empirical expressions for RET. Figure 2 is a plot of the ratio of acceptor to donor fluorescence at different ratios of chromophores as a function of the mean distance between chromophores. As the distance increases, the ratio of fluorescence tends to a limiting value irrespective of the ratio of chromophores. This value is presumably characteristic of the shape of the donor fluorescence spectrum and any direct excitation of the acceptor and corresponds to the absence of RET. The existence of this limiting value enables one to set an empirical limit beyond which RET does not occur. Furthermore, the fluorescence ratio is more sensitive to changes in distance between fluorophores at high ratios of acceptor to donor. Thus for our experiments the ratio of acceptor to donor was set at about 5 to 1. Clearly at some still higher acceptor to donor ratio, RET from donor to acceptor will be essentially complete over a wide range of distances. Consequently RET gives poor resolution of donor to acceptor distances at both very low and very high ratios.

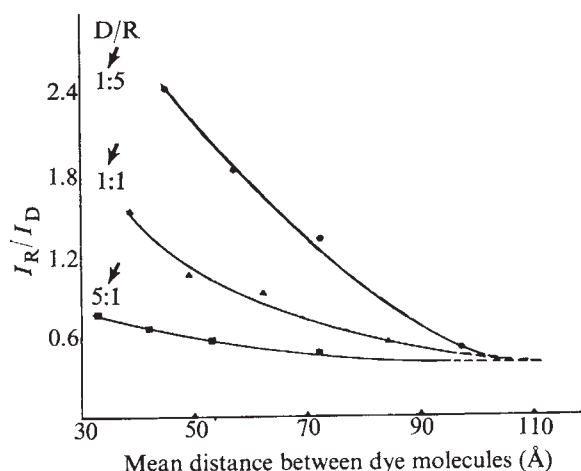


Fig. 2 Ratio of acceptor (rhodamine) to donor (DANS) fluorescence, I_R/I_D , in aggregated tubulin mixtures as a function of interfluorophore distance. The peak intensities at 570 nm and 515 nm were taken as a measure of acceptor and donor fluorescence, respectively. Tubulin was prepared by cyclic polymerisation by the method of Shelanski¹³ and labelled with DANS or RITC. Ratios of dye to protein were determined from the characteristic absorption at 350 nm and 553 nm, respectively, and protein by the method of Lowry¹⁴ using bovine serum albumin as standard. The separately labelled tubulin was mixed to give the desired acceptor to donor ratio and diluted with unlabelled tubulin in various amounts. The mixtures were then incubated with 20% glutaraldehyde in the cold overnight, precipitated with acetone and washed in media. The cross-linked precipitate was then smeared on a glass slide under a coverslip and examined in our instrument. The mean distance is calculated between all species of dyes taking each fluorophore as a point source and the density of the hydrated precipitate as 1.0.

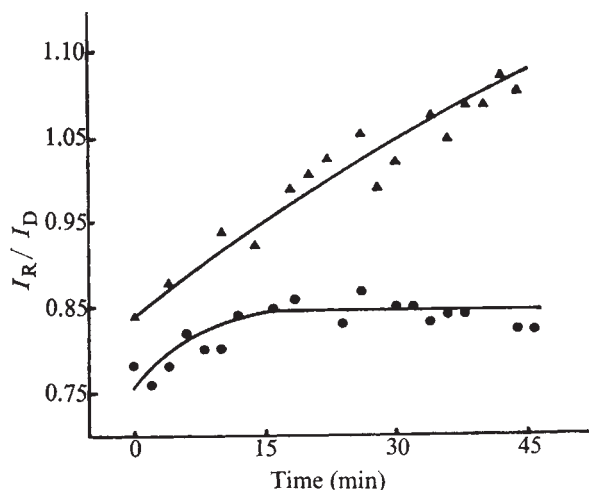


Fig. 3 Ratio of acceptor (rhodamine) to donor (DANS) fluorescence, I_R/I_D , from con A-labelled murine fibroblasts as a function of time of incubation at room temperature. The ratio of rhodamine to DANS was 5 to 1, the ratios of dye to con A protein were 3.8 to 1 for DANS-con A and 7 to 1 for RITC-con A. Total con A labelling concentration was $180 \mu\text{g ml}^{-1}$. Cells were labelled at 4°C for 1 h as described and excess dye was removed. Observations on the microscope stage at room temperature were commenced immediately after removal of dye. Cells were located by phase contrast using green filtered light. The flash lamp used to excite the specimen did not bleach the dye. In fixed specimens identical readings could be obtained for hours. Thus, membrane or other damage which could result from the high light intensities associated with bleaching (that are obviously essential for the photobleaching technique)²⁴⁻²⁶ is minimised. Δ , SV3T3 cells. $\bullet$, 3T3 cells.

Surface topography of con A-labelled fibroblasts

The application of the technique to living 3T3 and SV40-transformed 3T3 fibroblasts is partly confirmatory of previous studies of con A surface topography: C-R complexes are more clustered on transformed than normal cells^{4,5}. In addition, several features of the dynamics of C-R interaction are revealed, and the distribution of C-R complexes on 3T3 is shown to be non-random.

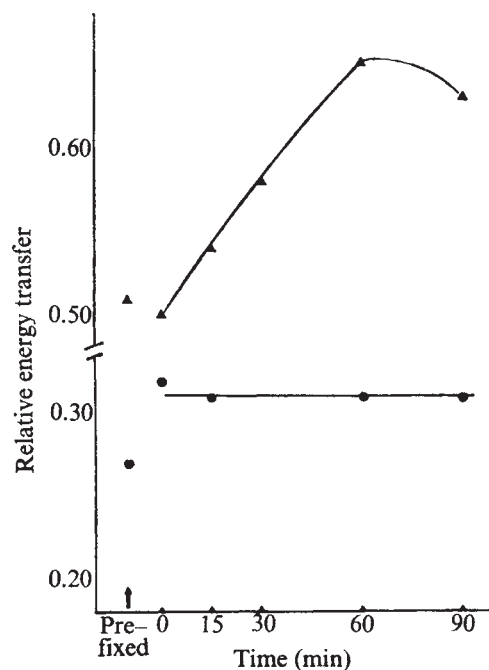
Con A was labelled with DANS (as the cycloheptaamylose complex)¹⁵ or RITC and purified by affinity chromatography as previously described¹⁶. Fibroblasts were grown in modified Dulbecco's medium with 10% calf serum on glass coverslips. Monolayers were labelled with mixtures of DANS-con A and RITC-con A at 4°C for 1 h. After rinsing away excess dye, the coverslips (fixed or unfixed) were inverted, sealed with paraffin on to glass slides and transferred to the instrument microscope stage. Cell-associated fluorescence was observed at intervals during incubation at room temperature. Data from single representative 3T3 and SV3T3 fibroblasts are shown in Fig. 3. Both cell types show an increase in RET with incubation. The relative change on 3T3, however, is quantitatively smaller than on SV3T3, and is completed after 15 min of incubation. We conclude that the topographical distribution of C-R complexes on SV3T3 is not random but also that the distribution on 3T3 is not completely random or disperse (although quantitatively the complexes are less clustered than for SV3T3). C-R complexes undergo a limited and rapid approximation on 3T3. By including large segments or even the entire cell in the field the average behaviour of C-R complexes over the whole region is recorded. Because with prolonged culture 3T3 spontaneously lose contact inhibition, we also studied C-R complexes on primary mouse fibroblasts and obtained results identical to those with our strain of 3T3.

Figure 4 shows an experiment in which the monolayers were fixed with paraformaldehyde after incubation at 37°C for

various times. The fixation allowed longer periods for accumulation of fluorescence photons and so there is less scatter of the data points. The same basic features are observed, indicating that fixation did not alter the topography of C-R complexes. At 37°C the aggregation of C-R complexes on 3T3 fibroblasts is completed by the time the first observation can be made at "0" min. But when cells are prefixed and then labelled, the RET obtained shows clearly that the time required for labelling at 4°C and subsequent fixation has allowed significant approximation of C-R complexes. Figure 4 again shows that the relative change of RET on SV3T3 is large but that the change occurs over 1 h even at 37°C .

An alternative method for estimation of RET is based on measurement of the lifetime of the excited state. RET depletes the population of excited donors and thus shortens the lifetime. If a nanosecond flash lamp is used as the exciting source, the lifetime of the excited state can be determined for the label on single cells. The techniques for data collection and analysis have been detailed elsewhere¹⁷. The results obtained from fixed preparations using this method agree qualitatively with the spectral data. Unfortunately, each determination of lifetime requires many minutes of photon counting so that the method was not suitable for observations of living cells nor for surveying large numbers of cells. Additional information on the distribution of bound con A, however, can be obtained from analysis of the fluorescence spectra taken at intervals during the lifetime of the excited donor population (so-called time-resolved spectra). For donors and acceptors separated by statistically variable distances the probability of RET between a donor and the population of acceptors that surround it will also vary. (This is in contrast to the situation in which donor and acceptor are separated by a fixed distance on the same molecule.) Consequently, immediately after excitation the probability of RET will be very high between close donors and acceptors and will decline rapidly as the remaining donors are separated from acceptors by greater distances. In Fig. 5 time resolved spectra from SV3T3 are recorded. The peak at 515 nm corresponds to DANS (donor) emission and decays with time as the excited state is depleted. The second peak at 570 nm results from the

Fig. 4 Ratio of acceptor to donor fluorescence, I_R/I_D , from con A-labelled cells against time. Conditions were as for Fig. 3, except that cells were fixed with 2% paraformaldehyde after incubation but before measurements of fluorescence. Incubation was at 37°C . "Prefixed" specimen was fixed for 20 min at 37°C before labelling. Δ , SV3T3 cells; $\bullet$, 3T3 cells.



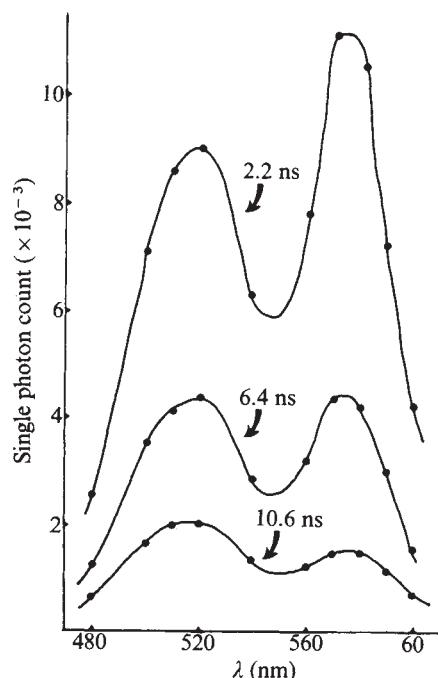


Fig. 5 Time resolved spectra from con A (DANS, rhodamine)-labelled SV3T3 fibroblasts. Photon counts were accumulated at various indicated times after the peak of the light pulse used for excitation. At 2.2 ns RET is high between DANS and nearby RITC. The DANS fluorophores that remain excited at later times are those which are statistically distributed at greater distances from the RITC and thus are less efficient in RET.

rhodamine fluorescence. The relative peak heights of fluorescence, rhodamine/DANS (acceptor/donor) are greatest early during the lifetime of the excited state in accord with the statistical surface distribution of the labelled con A.

The contribution of internalised (pinocytosed) con A to our measurements is probably not significant. We assessed the internalisation by determining the amount of con A that could not be eluted by the competing hapten sugar, α -methylmannoside. For these purposes radioactive (125 I-labelled) con A was used, for at intervals of less than 60 min the amount of internalised fluorescent label gave unreliably low signals. The amount of radioactivity that could not be eluted from dense monolayers in the conditions of our experiments was <10% of the total.

Inherent distribution of con A receptors

In agreement with observations made using other techniques, the approximation of receptors on SV3T3 is significantly greater than on non-transformed 3T3 or primary mouse fibroblasts^{4,5}. In addition, we find that the RET on prefixed SV3T3 is consistently higher than for prefixed 3T3 (example in Figs 3 and 4). This could be because at the high level of resolution afforded by the technique con A receptors are inherently more clustered on SV3T3, that is some of the approximation of C-R complexes on SV3T3 is not induced by the con A. Such an interpretation runs contrary to a large body of evidence using other techniques, indicating the inherent distribution to be disperse on all cell types but it should be emphasised that the degree of resolution afforded by RET exceeds that available by other techniques. Alternatively the surface density of con A receptors on SV3T3 may be higher than for 3T3. Note that this interpretation does not depend on measurements of the actual surface area. This conclusion was originally suggested by Inbar and Sachs¹⁸ and Noonan and Burger¹⁹ but has been difficult to test because of complications in the measurement of cell surface area. Using scanning electron microscopy, Collard and Temmink³ have attempted to account for variations in surface contour and

calculated a significantly greater density of con A receptors on SV3T3.

At the high resolution afforded by RET the topographical distribution of C-R complexes is not random on non-transformed 3T3 or primary mouse fibroblasts. Using the haemocyanin-con A and ferritin labelling techniques, we and others^{4,5} have reported the distribution of C-R complexes to be disperse or random. This conclusion was inferred largely from differences between normal and the more obvious clustered C-R aggregates on transformed mouse fibroblasts. Other groups have not found consistent differences between normal and transformed murine cells²⁰⁻²². Differences have been attributed to variations in cell line and to methodology. Our findings with RET do not completely resolve the issue since the high resolution obtained with RET cannot be compared directly with the topographical descriptions obtained by other techniques. In addition RET provides semiquantitative information integrated over large areas of the surface. Our results, however, suggest that topographical differences between normal and transformed murine fibroblasts are essentially quantitative.

Movement of C-R complexes

In agreement with previous observations involving C-R movement over long distances^{6,23} the non-randomness observed by RET on all cell types is induced to some degree by the con A itself, for prefixed cells or early time points show lesser degrees of RET.

The aggregation of C-R complexes on 3T3 cells is completed in a relatively short time compared with SV3T3 cells. Whether this is because the end point of aggregation represents a lesser degree of organisation or because the organisation is attained more efficiently in the 3T3 cannot be determined from the data reported here.

Quantitative aspects of this slow movement imply that the organisation of C-R complexes observed by RET is not limited by their lateral diffusion over the surface. We re-emphasise that the changes of RET observed require movements of only Ångströms, perhaps 100 Å at the most. A rough calculation of the diffusion coefficient (D) for C-R complexes can be obtained from our data using a well known expression relating the relaxation time of diffusion (τ) to the mean distance (ΔX) travelled by the complexes ($\Delta X^2 = 2\tau D$). For these purposes we can take τ as the time required to attain plateau levels of RET and ΔX to be between 10 and 100 Å. The range of D can then be calculated to be between 10^{-16} and 10^{-17} cm² s⁻¹. This is exceedingly small compared with all previous measurements of movements of antigen-antibody complexes or of succinyl-con A-receptor complexes. For example, experiments²⁴⁻²⁶ utilising the recovery of fluorescence after photobleaching have yielded diffusion coefficients of the order of 10^{-10} cm² s⁻¹ for succinyl con A on fibroblasts (even this is considerably lower than rhodopsin in photoreceptor membranes, 4×10^{-9} cm² s⁻¹). It is interesting that in the experiments of Jacobson *et al.*²⁵ and Schlessinger *et al.*²⁶ recovery from photobleaching was not complete in minutes and suggested a subpopulation of "immobilised" receptors. Even if we assume that the true diffusing species is a con A-cross-linked aggregate involving all bound con A (approximately 10^7 molecules per cell) the diffusion rate, which varies as the square root of the molecular weight, could not be lowered by 6 to 7 orders of magnitude. We suppose instead that the change in RET is not diffusion limited, but represents a secondary organisational change that involves some other mechanism, perhaps the interaction of submembranous structures (microtubules, microfilaments or spectrin-like molecules) with the cross-linked C-R complexes.

Future applications

The extension of RET to the study of the distribution of other surface constituents is obvious. We have reduced the analysis to an empirical semiquantitative one which provides considerable information without forcing assumptions or extensive instrumental corrections that are unreliable and cumbersome. Thus

the method provides a relatively straightforward and reproducible means for obtaining information on distances separating any exogenous ligand that can be labelled with donor and acceptor fluorophores without loss of activity. Distribution can in general be studied in living or fixed material. We have used average signals from large areas of the cell surface. Similar data, however, can be obtained from areas of the surface of as little as 2 μm in diameter, and so regional surface differences can also be compared.

The results obtained with con A serve to direct attention away from the concept of simple unrestricted mobility of surface complexes in the membrane lipid matrix. We predict that RET studies with other ligand-receptor complexes will support the view that cell-surface topography is determined to a large degree by subtle interactions between linked receptors and changes in submembranous elements induced by them.

This work was supported by grants from the NIH.

Received August 16; accepted October 19, 1976.

- ¹ Ozanne, B., and Sambrook, J., *Nature new Biol.*, **232**, 156–160 (1971).
² Cline, M. J., and Livingston, D. C., *Nature new Biol.*, **232**, 155–156 (1971).

- ³ Collard, J. G., and Temmink, J. H. M., *J. Cell Sci.*, **19**, 21–32 (1975).
⁴ Nicolson, G. L., *Nature new Biol.*, **233**, 244–246 (1971).
⁵ Rosenblith, J. Z., Ukena, T. E., Yin, H. H., Berlin, R. D., and Karnovsky, M. J., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 1625–1629 (1973).
⁶ Ukena, T. E., Borysenko, J. Z., Karnovsky, M. J., and Berlin, R. D., *J. Cell Biol.*, **61**, 70–82 (1974).
⁷ Förster, T. H., *Disc. Faraday Soc.*, **27**, 7–17 (1959).
⁸ Becker, J. S., Oliver, J. M., and Berlin, R. D., *Nature*, **254**, 152–154 (1975).
⁹ Stryer, L., *Science*, **162**, 526–533 (1968).
¹⁰ Steinberg, I. Z., and Katchalski, E., *J. chem. Phys.*, **48**, 2404–2410 (1968).
¹¹ Dale, R. E., and Eisinger, J., *Proc. natn. Acad. Sci. U.S.A.*, **73**, 271–273 (1976).
¹² Schiller, P. W., in *Biochemical Fluorescence: Concepts*, 1 (edit. by Chen, R. F., and Edelhoch, H.), 285–303 (Dekker, New York, 1975).
¹³ Shelanski, M. L., Gaskin, F., and Cantor, C. R., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 765–768 (1973).
¹⁴ Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., *J. biol. Chem.*, **193**, 265–275 (1951).
¹⁵ Kinoshita, T., Jinuma, F., and Tsuji, A., *Analyt. Biochem.*, **61**, 632–637 (1974).
¹⁶ Oliver, J. M., Zurier, R. B., and Berlin, R. D., *Nature*, **253**, 471–473 (1975).
¹⁷ Yguerabide, J., in *Methods in Enzymology*, **26**, part C (edit. by Hirs, C. H. W., and Timasheff, S. N.), 498–578 (Academic, New York, 1972).
¹⁸ Inbar, M., and Sachs, L., *Nature*, **223**, 710–712 (1969).
¹⁹ Noonan, K. D., and Burger, M. M., *J. biol. Chem.*, **248**, 4286–4292 (1973).
²⁰ Smith, S. B., and Revel, J.-P., *Devl Biol.*, **27**, 434–441 (1972).
²¹ Petris, S. de., Raff, M. C., and Mallucci, L., *Nature new Biol.*, **244**, 275–278 (1973).
²² Temmink, J. H. M., Collard, J. G., Spits, H., and Roos, E., *Expl Cell Res.*, **92**, 307–322 (1975).
²³ Nicolson, G. L., *Nature new Biol.*, **243**, 218–220 (1973).
²⁴ Zagayansky, Y., and Edidin, M., *Biochim. biophys. Acta*, **433**, 209–214 (1976).
²⁵ Jacobson, K., Wu, E., and Poste, G., *Biochim. biophys. Acta*, **433**, 215–222 (1976).
²⁶ Schlessinger, J., et al., *Proc. natn. Acad. Sci. U.S.A.*, **73**, 2409–2413 (1976).
²⁷ Poo, M.-M., and Cone, R. A., *Nature*, **247**, 438–441 (1974).

Crystallographic structure studies of an IgG molecule and an Fc fragment

Robert Huber, Johann Deisenhofer, Peter M. Colman* & Masaaki Matsushima

Max-Planck-Institut für Biochemie, 8033 Martinsried, BRD and Physikalisch-Chemisches Institut der Technischen Universität München

Walter Palm

Institut für Medizinische Biochemie der Universität Graz, Austria

The crystal structures of a human IgG antibody molecule Kol and a human Fc fragment have been determined at 4-Å and 3.5-Å resolution respectively, by isomorphous replacement. The electron-density maps were interpreted in terms of immunoglobulin domains based on the Rei and McPC 603 models (Kol) and by model-building (Fc). The Fab parts of Kol have a different quaternary structure from that observed in isolated crystalline Fab fragments, there being no longitudinal V-C contact in Kol. The Fc part C terminal to the hinge is disordered in the Kol crystals. It is suggested that the Kol molecule is flexible in solution, whereas fragments are rigid. In the Fc fragment both C_H3 and C_H2 show the immunoglobulin fold. The C_H3 dimer aggregates as C_H1-C_L while C_H2 are widely separated from each other. The carbohydrate bound to Fc is in fixed position. From these structures a hypothetical liganded antibody molecule has been constructed, which is assumed to be rigid.

ANTIBODY molecules serve a dual function: to recognise foreign cells and macromolecules and to trigger the events leading to their elimination. Specific recognition requires surface structures complementary to the antigen and hence a huge variety of antibody molecules. In contrast the effector functions would most economically be performed if all antibody molecules were identical. An antibody molecule of the IgG class consists of two identical light (L) and two identical heavy (H) chains^{1,2}. Each of these polypeptide chains can be subdivided into a constant

region with an amino acid sequence identical to other chains of the same class and a variable region whose sequence varies between antibodies specific for different antigens. Antibody functions are located on different parts of the molecule with recognition lying in a fragment consisting of a light chain and half of the heavy chain—the Fab fragment. Effector function on the other hand resides in the C-terminal half of the heavy chain—the Fc part. There is, however, cooperativity, and effector functions are triggered by antigen binding. How this signal is transferred is open to debate and in this article we will discuss a hypothetical model based on our current knowledge of three-dimensional antibody structure.

Several crystal structures of immunoglobulin fragments (Fab fragments and Bence-Jones proteins) have been analysed during the last few years (for a review, see ref. 3). From the recent crystal structure analyses of a complete human IgG antibody Kol⁴ and a human Fc fragment^{5,6} a coherent view of antibody structure is beginning to emerge.

The crystallographic analysis of the Kol protein was pursued to 5-Å resolution by isomorphous replacement⁴ and later extended to 4-Å resolution. The electron-density map was analysed in terms of domain structure making use of the known structure of the V (variable) and C (constant) parts of the Fab fragment McPC 603³ and the Rei fragment⁷. It was evident that there are differences in domain tertiary structure between the Kol protein and McPC 603. We have not yet tried to analyse these in detail, but it is clear that they do not affect the later discussion on longitudinal domain contracts. A tentative interpretation in the 4-Å map of the C^α chain folding is given for the heavy chain from residue 213 to the -Cys-Pro-Pro-Cys-Pro segment, and for the light chain from residue 209 to the heavy-light chain disulphide (amino acid sequences in the Kol Fab part refer to the McPC 603 molecule³ and to the Rei protein V_L dimer^{8–10}). The Fc fragment crystal structure was

*Present address: Institute of Inorganic Chemistry, University of Sydney 2060, Australia.

L

R

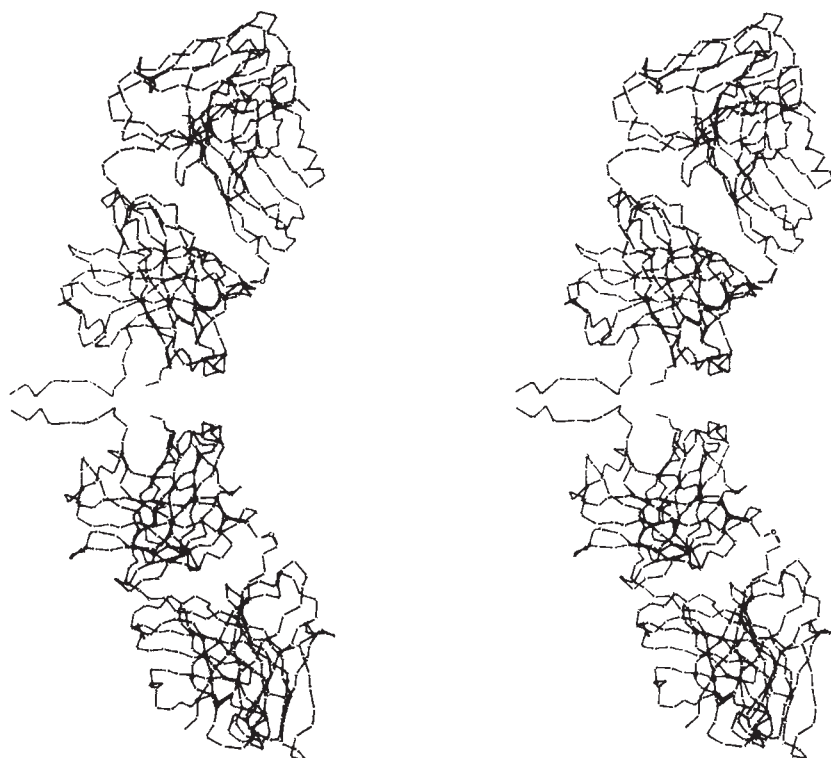


Fig. 1 Stereo drawing of the Kol (Fab)₂ part C α ; atom positions were derived by fitting the McPC 603 (ref. 3) V and C dimers to the Kol electron-density map. The coordinates of the C-terminal residues of heavy and light chains and the hinge peptide were obtained by interpretation of the 4-Å Fourier map.

Kol

analysed at 4-Å resolution⁵ and later at 3.5-Å resolution⁶, using isomorphous phases and a complete model was constructed.

The Kol molecule

The C α carbon atom positions of the (Fab)₂ part of the Kol molecule are shown in the stereogram, Fig. 1. The Fab arms subtend an angle of 125°, and the tips of the Fab arms are about 146 Å apart. Except for slight differences in the domain tertiary structure, the V_L-V_H and C_L-C_{H1} dimers appear to be indistinguishable between Kol and McPC 603. In particular, the geometry of aggregation within V and C dimers is very similar. There is a difference, however, in the relative orientation of V and C dimers which can be described by a rotation about an axis through the switch peptides at residues 118–119 (heavy chain) and 109–110 (light chain) (Fig. 2). (Switch peptides are the segments connecting V and C parts comprising residues at 110 (light chain) and 119 (heavy chain).) This, of course, requires a conformational change in the switch peptide segment, which might be accomplished with minimal perturbation as the segment is in an extended conformation. Figure 2 compares the McPC 603 Fab fragment and the Kol Fab part as seen down the switch peptide rotation axis. V_L and V_H, as well as C_L and C_{H1} are related by local diads. These diads subtend an angle (Fab angle) of 135° in the McPC 603 fragment and 170° in the Kol protein, demonstrating the major change in quaternary structure: 'bending the elbow'.

It is already evident from Fig. 2 that in the McPC 603 molecule some residues of V_H and C_{H1} come close to each other. There are 6 C α -C α distances smaller than 6.5 Å (formed by segments 8–10 in V_H and 152 and 207–208 in C_{H1}) and 41 below 10 Å. There are, of course, several contacts between residues of the switch peptide and C_{H1} not included. We believe that these are irrelevant for the later discussion. Equivalent residues of V_L C_L of the light chain adopting the heavy chain conformation are in contact in the Bence-Jones protein dimer structure (ref. 11 and A. Edmundson, personal communication). In contrast, due to the asymmetry of the molecule, V_L and C_L are much further separated in the McPC 603 molecule. There are 2 C α distances below 6.5 Å and 28 below 10 Å.

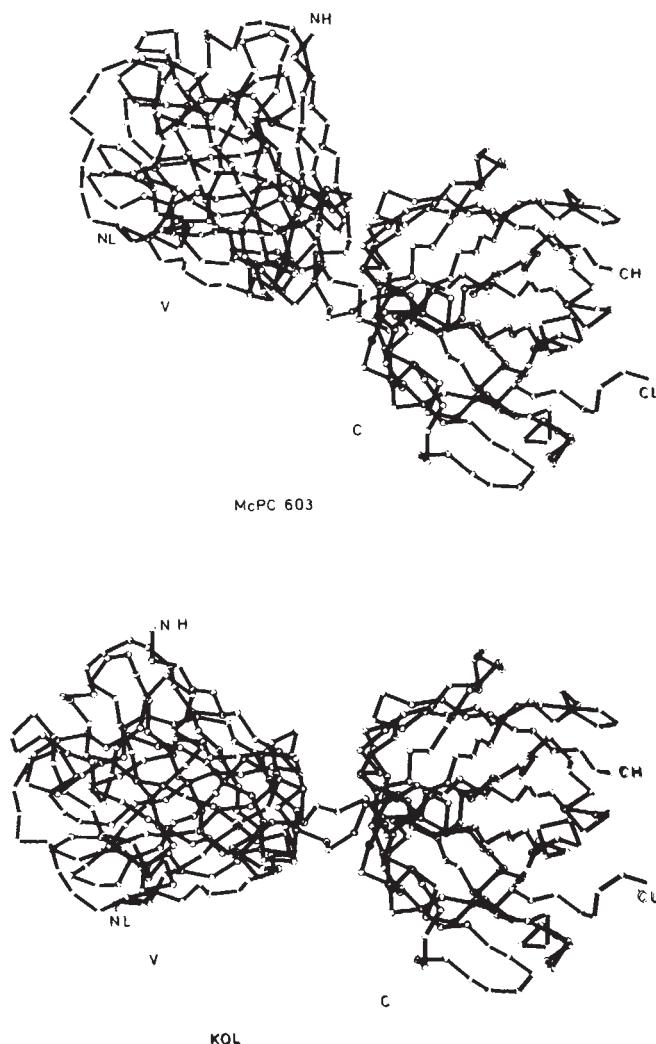
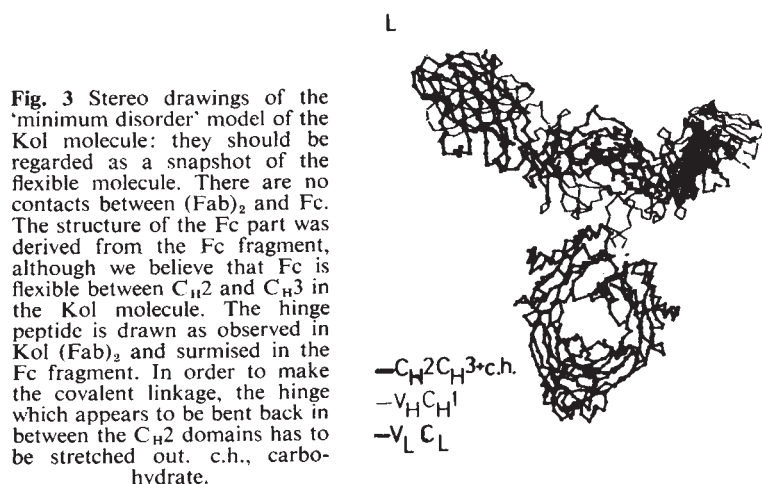
Through the widening of the Fab angle from 120° to 170° in Kol there is no contact between C α atoms of V_H and C_{H1} smaller than 7 Å. Five distances are below 10 Å. Also, V_L and C_L have no contact (six distances below 10 Å). For comparison, the lateral V_L-V_H and C_L-C_{H1} contacts in the McPC 603 Fab fragment involve nine and seven C α distances respectively closer than 6.5 Å and about 120 distances below 10 Å for both contacts. The C_{H3}-C_{H3} contact in Fc involves 14 C α distances smaller than 6.5 Å and about 150 below 10 Å. According to this admittedly very crude criterion, there is a strong V_H-C_{H1} interaction in crystalline Fab fragment structures while there is no appreciable longitudinal interaction in the Kol Fab parts. In rabbit light chains a disulphide bond links V_L and C_L residues 80 and 172 (ref. 12). A sufficiently close approach in the Kol model occurs between C α atoms of residues 81 and 171 of 9 Å. This distance is 7 Å in the McPC 603 model. The disulphide linkage therefore does not oppose the Fab angle ('elbow') bending considerably. The two Fab arms in the Kol protein have no contact with each other except the covalent linkage of the hinge. There is no approach of C α of the two C_L domains closer than 12.5 Å.

No interpretable electron density is found in the Kol Fourier map which could be assigned to the Fc part⁴. The Fourier map contains significant density in that region where the Fc stem must be located with peak heights about 2.5 times the r.m.s. error in electron density. This is to be compared with the density in the Fab parts with peak heights about four times the error level. We have concluded that the Fc stem is disordered in the Kol crystals, C-terminal to the hinge peptide. Indeed, the (Cys-Pro-Pro-Cys)₂ peptide of the hinge on the molecular and crystallographic diad is only 50 Å away from the crystallographic 3₂ axis. The Fc fragment molecule is too long and too thick to be accommodated on the diad axis^{4,6}. It would interfere with crystallographically related Fc parts. On the other hand, the very close packing in the Kol crystal structure around the hinge peptide (see Fig. 6 in ref. 4) does not allow a close approach of the C_{H2} domains of Fc towards (Fab)₂, but requires a rather extended hinge segment (the hinge peptide is the segment connecting C_{H1} and C_{H2} and containing the inter-heavy chain disulphide linkage).

We constructed a 'minimum disorder' model of the Kol molecule. The Fc fragment structure, when combined with the Fab parts in the Kol crystal structure, should deviate minimally from the Kol crystal symmetry and not violate the crystal packing. The Fc fragment model was placed with its local diad on the crystallographic diad of the Kol lattice so that the hinge peptide, as seen in the Kol molecule and as surmised in the Fc fragment (see later), overlapped. Fc was then rotated azimuthally so that there was minimal overlap with (Fab)₂ parts from crystallographically related molecules. It had to be shifted by about 15 Å along the diad, away from the Fab parts, to avoid overlap. Here it would penetrate the 3₂ axis and interfere with crystallographically related Fc parts. Fc was therefore bent about an axis close to the top of the molecule and perpendicular to the a b plane of the Kol lattice minimally, to avoid this steric interference. It is obvious that these three operations are not independent. Apparently, a correct but intractable three-dimensional treatment would not change the main aspect of the model in a substantial way. Figure 3 represents the model. Evidently there are no contacts between (Fab)₂ and Fc, except the covalent linkage through the hinge peptide, which must have a rather extended conformation. In the 'minimum disorder' model the wide separation of (Fab)₂ and Fc requires this piece of chain to be stretched out. These observations of absent longitudinal interactions between V_H and C_H1 and between C_H1 and C_H2, as well as missing lateral interactions between both C_L parts in the Kol molecule in comparison to the existing V_H-C_H1 interactions in free Fab fragments, will be important in later discussion.

Fc fragment structure

Figure 4 is a stereo plot of the C^α carbon atom positions of the Fc fragment. The molecule has the shape of a Mickey Mouse with the compact C_H3 dimer forming the head, and the C_H2 domains forming the ears. The C_H3 dimer pairing is closely similar to the C_H-C_L pairs found in Fab fragments^{5,6}. C_H2 and C_H3 show the common immunoglobulin fold. They are connected by a loosely folded segment from Ser (337) to Gln (342) which is susceptible to proteolytic attack^{13,14} (amino acid sequence numbers in the Fc fragment are based on the Eu amino acid sequence¹). The longitudinal C_H2-C_H3 contact is characterised by 3 C^α distances less than 6.5 Å (and 43 below 10 Å), not considering contacts made by the connecting peptide. It comprises the segments 247-253 and 310-314 (C_H2) and 376-379 and 428-433 (C_H3) respectively. These segments are homologous to those mediating the V_H-C_H1 contact in Fab fragments described before. The electron density at the C terminus fades away after Ser (442), indicating mobility from this residue on. The N terminus seems to be folded back from Pro (238) on and in intimate contact with the carbohydrate moiety discussed below. This mostly unresolved contact makes an interpretation of the peptide chain conformation around the hinge region difficult at present. A possible chain tracing has



the segment from Gly (236) to Pro (232) in contact with one branch of the carbohydrate chain. The (Cys-Pro-Pro-Cys)₂ segment seems to be disordered. This is not unexpected, as in the Kol crystals the (Cys-Pro-Pro-Cys)₂ segment is fixed while the Fc part is disordered. The electron density assigned

the segment from Gly (236) to Pro (232) in contact with one branch of the carbohydrate chain. The (Cys-Pro-Pro-Cys)₂ segment seems to be disordered. This is not unexpected, as in the Kol crystals the (Cys-Pro-Pro-Cys)₂ segment is fixed while the Fc part is disordered. The electron density assigned

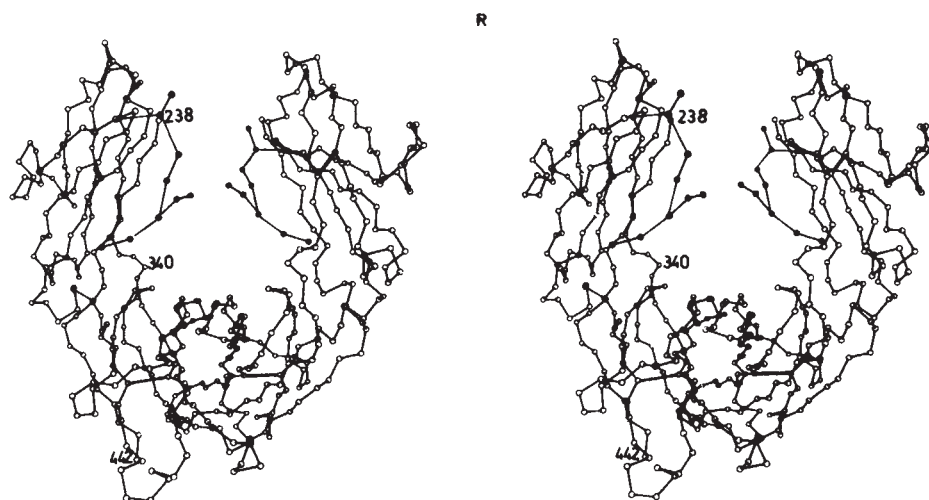
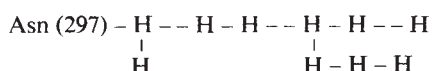


Fig. 4 Stereo drawing of the C^α carbon positions and the centres of the carbohydrate hexose units of the Fc fragment. The carbohydrate structure was assumed as described in the text. ●, Approximate centres of carbohydrate hexose units. The carbohydrate attachment site is Asn (297). ○, C^α carbon positions. The disulphide linkages are indicated.

to the bound carbohydrate could accommodate a branched carbohydrate chain of the following arrangement.



(H is a hexose unit; at three positions (—) we had difficulties in keeping linked residues in density. The present model is not connected at these positions.)

Such a chain would differ from some carbohydrate sequences of myeloma proteins¹⁵. We have to expect inhomogeneity of the carbohydrate in our material which might make the detailed analysis difficult. The crystallographic analysis of the carbohydrate structure requires further structure refinement. The carbohydrate moiety covers a large part of the C face of the C_{H2} domain.

Our model indicates that the carbohydrate covers apolar residues on the C face, among these being: Phe (241), Phe (243), Val (262), Val (264), Val (266). The site of attachment of the carbohydrate moiety at Asn (297) is part of a reverse turn. The four residues in the turn are Tyr-Asn-Ser-Thr. In general, carbohydrate attachment preferentially occurs at amino acid sequences X-Asn-X-Thr(Ser) (see review, ref. 16). Pancreatic trypsin inhibitor (PTI) and ribonuclease are molecules of known three-dimensional structures¹⁷⁻¹⁹. There exist closely related variants of these molecules (colostrum inhibitor²⁰ and ribonuclease B²¹) which carry carbohydrate. The carbohydrate site is part of a bend in all cases. The detailed conformations are very different, however.

Domain tertiary structure comparison

A set of comparative drawings of the individual domains in the Fab fragments McPC 603 and New, and the Bence-Jones proteins Mcg and Rei has been published³. A detailed comparison with the chain folding in the (Fab)₂ part of the Kol molecule has to await higher resolution data of the Kol crystals.

Comparative drawings of the chain folding of V, C_{H1} , C_{H2} and C_{H3} domains have been prepared and are shown in ref. 6. The most prominent differences between V and C domains lie in loops mediating V-V and C-C aggregation respectively. According to these criteria, it is clear that Fc C_{H3} has the characteristics of a C domain: the long loop involved in the C-C interface is present with the segment 398-403 forming the corner. V-characteristic loops are absent. C_{H2} , however, has some of the features of a V domain: the V characteristic loop is present, but shorter than in V domains. The C characteristic loop is also present, but shorter than in C domains and longer than in V domains. This raises the interesting question of the evolutionary origin of the C_{H2} segment. While amino acid

sequence homologies have been interpreted as indicating a close relation of C_{H2} to other C segments and only a distant relation to V segments, the structural comparison points to an intermediate structure. There is a remote possibility that the type of aggregation influences chain folding, the C_{H2} conformation being characteristic of a free domain, and the other C segments being deformed by their C-C contacts.

Domain-domain interactions

(1) Lateral interactions. The lateral V-V (V_H - V_L , V_L - V_L) and C-C (C_L - C_{H1} , C_L - C_L) interactions observed in Fab fragments^{22,23} and Bence-Jones proteins^{10,24} have been described in great detail and will not be considered here. The relative arrangement of the two C_{H3} domains observed in the Fc fragment is closely similar to the C_L - C_{H1} aggregation observed in McPC 603. The C_{H2} domains have no contact with each other except the covalent linkage through the hinge. It is an intriguing question why C_{H2} forms neither the familiar C-C nor V-V contact. It has been suggested that the tertiary structure of the C_{H2} domain does not resemble a C but also lacks some features of a V domain. This might prevent dimerisation. In the presence of the spatially fixed carbohydrate moiety formation of the C-C contact is impossible. Furthermore, some of the hydrophobic residues in C_{H3} , which lie close to the local diad, and are probably important for the contact, are replaced by polar groups in C_{H2} . An analogous argument holds against a potential V-V contact.

(2) Longitudinal interactions. In the Kol protein and the 'minimum disorder' model derived from it, no longitudinal contacts are observed between V_H and C_{H1} , V_L and C_L , and C_{H1} and C_{H2} . There are also no contacts between the Fab arms. In view of this lack of stabilisation along the chain, it is difficult to assume that the Kol molecule should be rigid in solution. We rather presume that flexibility exists between V and C dimers ('elbow' V-C flexibility), between the Fab arms ((Fab)₂ flexibility) and between Fc and (Fab)₂ (Fc-(Fab)₂ flexibility). As mentioned before, flexibility between Fc and (Fab)₂ could explain the weak, uninterpretable electron density for Fc in the Kol crystals. The relative positions of V and C dimers and Fab arms are frozen in the Kol crystal lattice dictated by crystal packing requirements. Even the C_{H2} segments might sit flexibly on the C_{H3} dimers (C_{H2} - C_{H3} flexibility) in the Fc part in the Kol protein in contrast to their fixed position in the fragment. We have, of course, no evidence for this as the Fc part in the Kol crystals is not yet analysed. Rigidity without longitudinal contact between the domains could only be brought about if the switch peptides and the hinge peptide adopted rigid conformations. These segments are in an extended conformation, largely exposed to solvent. Inherent rigidity of these peptide chains is improbable.

The model which we would like to propose for the Kol protein and for other unliganded IgG molecules is a highly flexible one.

The degree of segmental flexibility appears more limited for V-C ('rotation' about 'switch axis') than for (Fab)₂ or Fc-(Fab)₂, which are connected by far longer extended polypeptide chains.

In obvious contrast, the Fab fragments and the closely similar Bence-Jones protein dimer appear to be rigid molecules. In the three structures, V and C dimers subtend a similar Fab ('elbow') angle of 120° to 135°. Crystal packing is different in the three crystal structures and would be unlikely to have the same effect on the Fab angle if it were variable. Indeed, a longitudinal contact exists between V_H and C_H1 in these fragments as described before. This contact surface is smaller in size than the lateral C-C or V-V contacts, but it has been found in other systems that small contact surfaces may mediate strong binding (PTI-inhibitor-trypsin²⁵). In the Fc fragment C_H2 and C_H3 form a contact as described. The linking peptide is not in a completely extended conformation but is irregularly bent between residues Lys (338) and Gly (341). The presence of two nearly identical contacts in crystallographically non-identical environment speaks for a rigid Fc fragment, although it would be desirable to confirm this by structure analyses of different crystal forms.

It is remarkable and deserves further consideration that homologous segments mediate the close V-C contacts in the Fab fragment and the close C_H2-C_H3 contacts in the Fc fragment: V_H, residues 8-10; C_H1, residues 152 and 207-208 (McPC 603); C_H2, residues 247-253 and 310-314; C_H3, residues 376-379 and 428-433.

These contacts have been inferred from C^α distance calculations. The actual bonding interactions are, of course, made by side chains and/or polar atoms of the backbone. These segments contain residues highly conserved in immunoglobulin subclasses and classes. Of particular interest is the conservation of the three hydrophobic residues in C_H2 (Leu-Met-Ile (251-253)) in all IgG subclasses, but also in IgM C_H3 (Ile-Phe-Leu (352-354))²⁶ IgA C_H2 (Leu-Leu-Leu (256-258))²⁷ and IgE C_H3 (Leu-Phe-Ile (339-341))²⁸. We note in passing that this observation adds to the evidence that in IgM and IgE, which contain an additional constant domain, C_H3 resembles C_H2 in IgG. In C_H1 and C_H3 ('top' contact area) the contact residues are from the bends C-terminal to the two intra-domain Cys residues. In V_H and C_H2 ('bottom' contact area) these are predominantly residues of the bend N-terminal to the first intra-domain Cys residue. It is of further interest that the top contact segments described for C_H1 and C_H3, when projected into a V segment, coincide with the first and third hypervariable segments forming the antigen-binding surface in V dimers.

In an allosteric immunoglobulin model which we will propose in a later discussion, the signal transfer through the domains from the top contact to the bottom contact occurs along homologous segments in all domains.

The rigidity of Fab and Fc fragments and the segmental flexibility in the complete molecule Kol might just be a coincidence and future studies might provide a better statistical sample

of the whole population of molecules. The following model-building studies and discussion are based on the hypothesis that (1) rigidity and segmental flexibility are indeed inherent properties of fragments and complete, unliganded molecules, respectively, and (2) the rigid Fab fragment structures are characteristic for the liganded molecules. This is primarily based on the observation that Fab fragments show no gross structural change upon hapten binding^{22,23}. We are aware that different explanations for this observation are possible. In particular, hapten binding might be insufficient to produce structural changes brought about by antigen.

We constructed a hypothetical liganded antibody molecule by combining the fragment molecules. Starting with the Kol (Fab)₂, we set the Fab elbow angle to 120°, which is characteristic for Fab fragments. The hinge peptide, which is well defined in the Kol crystals and less well ordered—but apparently bent back—in Fc fragment crystals were superimposed with the local diads coinciding. Fc was then rotated azimuthally in order to avoid overlap between C_H1 and C_H2 of the same molecule. A narrow range of the azimuthal angle turned out to be possible. The resulting model is shown in Fig. 5. C_H1 and C_H2 are close at the bottom and top contact segments discussed before, which comprise conserved residues in immunoglobulin subclasses and classes. The hinge peptide forms numerous internal contacts with both domains and the carbohydrate. It is obvious that in view of the (Fab)₂ flexibility of the Kol molecule, the choice of the relative arrangement of the Fab arms was arbitrary. By a (Fab)₂ rearrangement the formation of a lateral contact between the Fab arms appears possible.

The important aspect of this model, which we want to emphasise, is that all longitudinal interdomain contacts are closed, resulting in a rigid structure. (Fab)₂ bending is prohibited by the C_H1-C_H2 interaction.

We suggest that antigen binding causes a stiffening of the flexible antibody molecule by formation of the longitudinal inter-domain contacts described. The largest structural change involves the folding of the hinge peptide in between the C_H2 globules to allow the C_H1-C_H2 approach. This model allows deletion of a large proportion of the hinge segment as observed in two crystalline antibodies^{29,30}, without change in the relative Fab Fc arrangement, in contrast to the flexible model with the extended hinge segment as described before. The hinge deletion forces C_H1 and C_H2 to form the contact. This could induce an overall quaternary structural change to the rigid conformation. The Fab bending produces a model more like a T compared to the Y of the Kol molecule (Figs 3 and 5). A T-shaped model has been suggested from low resolution X-ray studies of the myeloma protein Dob³¹. This protein has a hinge deletion²⁹. Electron micrographs of hapten cross-linked antibodies generally show a short Fc stem as we would expect from Fig. 5^{32,33}.

Segmental flexibility between Fab and Fc parts of antibody molecules is experimentally established by spectroscopic and hydrodynamic observations^{34,35}. Hydrogen-exchange experiments, as well as experiments with limited proteolysis of IgM and their Fab fragments, may be interpreted in terms of a less flexible fragment structure^{36,37}.

There are several observations of structural changes in

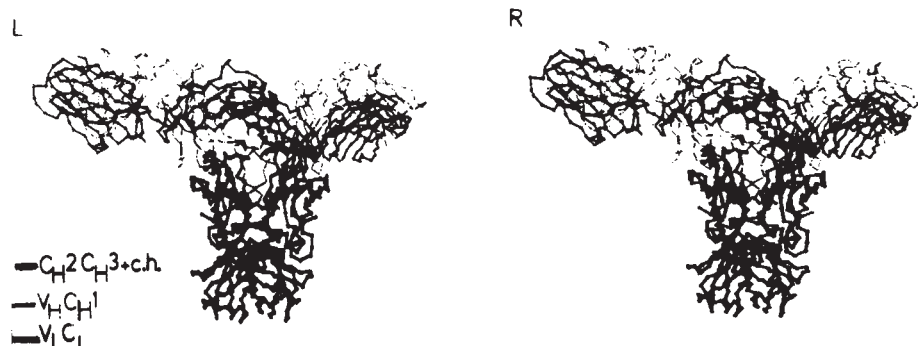


Fig. 5 Stereogram of a hypothetical liganded IgG molecule. The molecule is T-shaped with Fc forming the stem. Fc and (Fab)₂ are in close contact. The hinge peptide is folded back in between the C_H2 parts. c.h., carbohydrate.

antibody molecules upon antigen binding. Circular polarisation of fluorescence (CPL) changes in complete antibodies upon antigen binding, but not in Fab fragments^{38,39}. An antigen-induced volume contraction has been found by small angle X-ray scattering in complete antibodies, but not in Fab fragments^{40,41}. Antibodies seem to lose flexibility on antigen binding^{42,43} and to gain conformational stability against denaturation⁴⁴. The structural difference in the Fab part between complete antibody and Fab fragment ('elbow' bending) might manifest itself in a change of the characteristics of ligand binding. Observations of differences in rate constants of fragments and complete antibodies are controversial^{45,46}. There are, apparently, no significant hapten binding affinity differences between Fab fragments and complete antibodies, indicating that there is no cooperativity between the two hapten binding sites in an antibody molecule⁴⁷. This does not contradict the ligand-induced structural change proposed, as the energy required for the conformational change might be provided by an increased ligand affinity with both effects cancelling out. There are indeed observations on IgM molecules of differences in thermodynamic parameters for haptens and antigens⁴⁸, interpreted as indicating an antigen-induced structural change.

Before the X-ray structure determination of a liganded antibody molecule, there are experiments that could disprove or verify the hypothetical model of ligand induced conformational changes proposed. Chemical modification of residues participating in the longitudinal inter-domain contacts might help in determining the origin of the spectroscopic signal produced by ligand binding^{38,39}. The hinge peptide undergoes the most extensive local structural change in our model. This should be reflected in the kinetics of disulphide reduction or proteolytic cleavage. The crystal structure analysis of the Fab fragment Kol is under way in our laboratory. This molecule we would expect to be in the bent conformation. Detailed X-ray models of the IgG molecules Dob^{29,31} and Mcg^{30,49}, which have a deletion in the hinge-region, would be of great interest, as they might resemble liganded molecules.

We have used the PROTEIN program system written by Dr Wolfgang Steigemann in this laboratory. We thank Drs G. Schwick and H. Haupt, Behringwerke, Marburg, for providing the Fc material, Frau K. Epp for X-ray technical assistance and the Deutsche Forschungsgemeinschaft und Sonderforschungsverein 51 for financial assistance.

Received April 5; accepted October 11, 1976.

¹ Rutishauser, U., Cunningham, B. A., Bennet, C., Konigsberg, W. H., and Edelman, G. M., *Biochemistry*, **9**, 3171-3181 (1970).

² Fleischmann, J. B., Pain, R. H., and Porter, R. R., *Archs Biochem. Biophys. Suppl.*, **1**, 174 (1972).

- ³ Davies, D. R., Padlan, E. A., and Segal, D. M., *A. Rev. Biochem.*, **44**, 639-667 (1975).
- ⁴ Colman, P. M., Deisenhofer, J., Huber, R., and Palm, W., *J. molec. Biol.*, **100**, 257-282 (1976).
- ⁵ Deisenhofer, J., Colman, P. M., Huber, R., Haupt, H., and Schwick, G., *Hoppe-Seyler's Z. Physiol. Chem.*, **357**, 435-445 (1976).
- ⁶ Deisenhofer, J., Colman, P. M., Epp, O., and Huber, R., *Hoppe-Seyler's Z. Physiol. Chem.*, **357**, 1421-1434 (1976).
- ⁷ Epp, O., et al., *Eur. J. Biochem.*, **45**, 513-524 (1974).
- ⁸ Palm, W., and Hilschmann, N., *Hoppe-Seyler's Z. Physiol. Chem.*, **356**, 167-191 (1975).
- ⁹ Palm, W., *Hoppe-Seyler's Z. Physiol. Chem.*, **355**, 877-880 (1974); Palm, W., *Hoppe-Seyler's Z. Physiol. Chem.*, **357**, 795-789 (1976); Palm, W., *Hoppe-Seyler's Z. Physiol. Chem.*, **357**, 799-802 (1976).
- ¹⁰ Epp, O., Lattman, E. E., Schiffer, M., Huber, R., and Palm, W., *Biochemistry*, **14**, 4943-4952 (1975).
- ¹¹ Schiffer, M., Girling, R. L., Ely, K. R., and Edmundson, A. B., *Biochemistry*, **12**, 4620-4631 (1973).
- ¹² Strosberg, A. D., Fraser, K. J., Margolies, M. N., and Haber, E., *Biochemistry*, **11**, 4978-4985 (1972).
- ¹³ Connell, J. E., and Porter, R. R., *Biochem. J.*, **124**, 53P (1971).
- ¹⁴ Kehoe, J. M., Bourgeois, A., Capra, J. D., and Fougereau, M., *Biochemistry*, **13**, 2499-2504 (1974).
- ¹⁵ Kornfeld, R., Keller, J., Baenziger, J., and Kornfeld, S., *J. biol. Chem.*, **246**, 3259-3268 (1971).
- ¹⁶ Marshall, R. D., *A. Rev. Biochem.*, **41**, 673-702 (1972).
- ¹⁷ Huber, R., Kukla, D., Rühlmann, A., Epp, O., and Formanek, H., *Naturwissenschaften*, **57**, 389-392 (1970).
- ¹⁸ Kartha, G., Bello, J., and Harker, D., *Nature*, **213**, 862-865 (1967).
- ¹⁹ Wyckoff, H. W., et al., *J. biol. Chem.*, **242**, 3749-3753 (1967).
- ²⁰ Tschesche, H., Klausner, R., Cechová, D., and Yanáková, V., *Hoppe-Seyler's Z. Physiol. Chem.*, **356**, 7159-7164 (1974).
- ²¹ Jackson, R. L., and Hirs, C. H. W., *J. biol. Chem.*, **245**, 637-653 (1970).
- ²² Amzel, L. M., Poljak, R. J., Saul, F., Varga, J. M., and Richards, F. P., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 1427-1430 (1974).
- ²³ Segal, D. M., Padlan, E. A., Cohen, G. H., Rudikoff, S., Potter, M., and Davies, D. R., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 4298-4302 (1974).
- ²⁴ Edmundson, A. B., Ely, K. R., Abola, E. E., Schiffer, M., and Panagiotopoulos, N., *Biochemistry*, **14**, 3953-3961 (1975).
- ²⁵ Huber, R., Kukla, D., Bode, W., Schwager, P., Bartels, K., Deisenhofer, J., and Steigemann, W., *J. molec. Biol.*, **89**, 73-101 (1974).
- ²⁶ Watanabe, S., Barnikol, H. U., Horn, J., Bertram, J., and Hilschmann, N., *Hoppe-Seyler's Z. Physiol. Chem.*, **354**, 1505-1509 (1973).
- ²⁷ Kratzin, H., Altevogt, P., Ruban, E., Kortt, A., Staroscik, K., and Hilschmann, N., *Hoppe-Seyler's Z. Physiol. Chem.*, **356**, 1337-1432 (1975).
- ²⁸ Bennis, H., and Bahr-Lindström, H., *Progress in Immunology II*, **1** (edit. by Brent, L., and Holborow, J.), 49-58 (North-Holland, Amsterdam, Oxford, 1974).
- ²⁹ Lopes, A. D., and Steiner, L. A., *Fedn Proc.*, **32**, 1003 (1973).
- ³⁰ Felt, J. W., Deutsch, H. W., and Smithies, O., *Immunochimistry*, **10**, 115-118 (1973).
- ³¹ Sarma, V. R., Silverton, E. W., Davies, D. R., and Terry, W. D., *J. biol. Chem.*, **246**, 3753-3759 (1971).
- ³² Valentine, R. C., and Green, N. M., *J. molec. Biol.*, **27**, 615-617 (1965).
- ³³ Feinstein, A., and Rowe, A. J., *Nature*, **205**, 147-149 (1965).
- ³⁴ Yguerabide, J., Epstein, H. F., and Stryer, L., *J. molec. Biol.*, **51**, 573-590 (1970).
- ³⁵ Noelken, M. E., Nelson, C. A., Buckley, C. E., and Tanford, C., *J. biol. Chem.*, **240**, 218-224 (1965).
- ³⁶ Ashman, R. F., Kaplan, A. P., and Metzger, H., *Immunochemistry*, **8**, 627-641 (1971).
- ³⁷ Ashman, R. F., and Metzger, H., *Immunochemistry*, **8**, 643-656 (1971).
- ³⁸ Schlessinger, J., Steinberg, I. Z., Givol, D., Hochman, J., and Pecht, I., *Proc. natn. Acad. Sci. U.S.A.*, **72**, 2775-2779 (1975).
- ³⁹ Jaton, J. C., Huser, H., Braun, D. G., Givol, W., Pecht, I., and Schlessinger, J., *Biochemistry*, **14**, 5312-5315 (1975).
- ⁴⁰ Pilz, I., Kratky, O., Licht, A., and Sela, M., *Biochemistry*, **12**, 4998-5005 (1973).
- ⁴¹ Pilz, I., Kratky, O., Licht, A., and Sela, M., *Biochemistry*, **14**, 1326-1333 (1975).
- ⁴² Tumerman, L. A., Neelin, R. S., and Zagayanski, Y. A., *FEBS Lett.*, **19**, 290-292 (1972).
- ⁴³ Warner, C., and Shumaker, V., *Biochemistry*, **9**, 451-459 (1970).
- ⁴⁴ Cathou, R. E., and Warner, T. C., *Biochemistry*, **9**, 3149-3155 (1970).
- ⁴⁵ Kelly, K. A., Schon, A. H., and Froese, A., *Immunochimistry*, **8**, 613-625 (1971).
- ⁴⁶ Levison, S. A., Hicks, A. N., Portmann, A. J., and Dandliker, W. B., *Biochemistry*, **14**, 3778-3786 (1975).
- ⁴⁷ Nisonoff, A., Wissler, F. C., and Woernley, D. L., *Archs Biochem. Biophys.*, **88**, 241-245 (1960).
- ⁴⁸ Brown, J. C., and Koshland, M. E., *Proc. natn. Acad. Sci. U.S.A.*, **72**, 5111-5115 (1975).
- ⁴⁹ Edmundson, A. B., Schiffer, M., Wood, M. K., Hardman, K. D., Ely, K. R., and Ainsworth, C. F., *Cold Spring Harb. Symp. quant. Biol.*, **26**, 427-432 (1972).

letters to nature

Atmospheric gravity wave production for the solar eclipse of October 23, 1976

CHIMONAS and Hines¹ suggested that during a solar eclipse the cool shadow of the Moon moving with supersonic speed through the Earth's atmosphere should generate bow waves. Upper atmospheric disturbances, having quasi-periods of some 20 min, were recorded^{2,3} after the eclipse of March 7, 1970, and were regarded as possible verification of Chimonas and Hines's¹ suggestion. Other observations^{4,5} following the eclipse of June 30, 1973, failed to detect such disturbances. The eclipse of October 23, 1976, will provide another opportunity in attempting to record eclipse-generated atmospheric disturbances.

As a result of the curvature of the eclipse path and variations in speed of the Moon's shadow, focusing of the waves should occur at calculable positions and times^{6,7}. Enhanced amplitude in a focal region should provide optimal conditions for verifying the existence of the bow wave⁷. We have made calculations of the form of the bow waves expected for the eclipse of October 23, 1976, and in particular the positions and times at which focusing effects are expected, using the eclipse path calculated by Fiala and Duncombe⁸, and the technique outlined by Beer and May⁶. Figure 1 shows the calculated positions of the northwards and southwards moving bow wave-fronts, as plots of constant time, T . Ray paths lying along great circles, are also indicated for disturbances originating at time t .

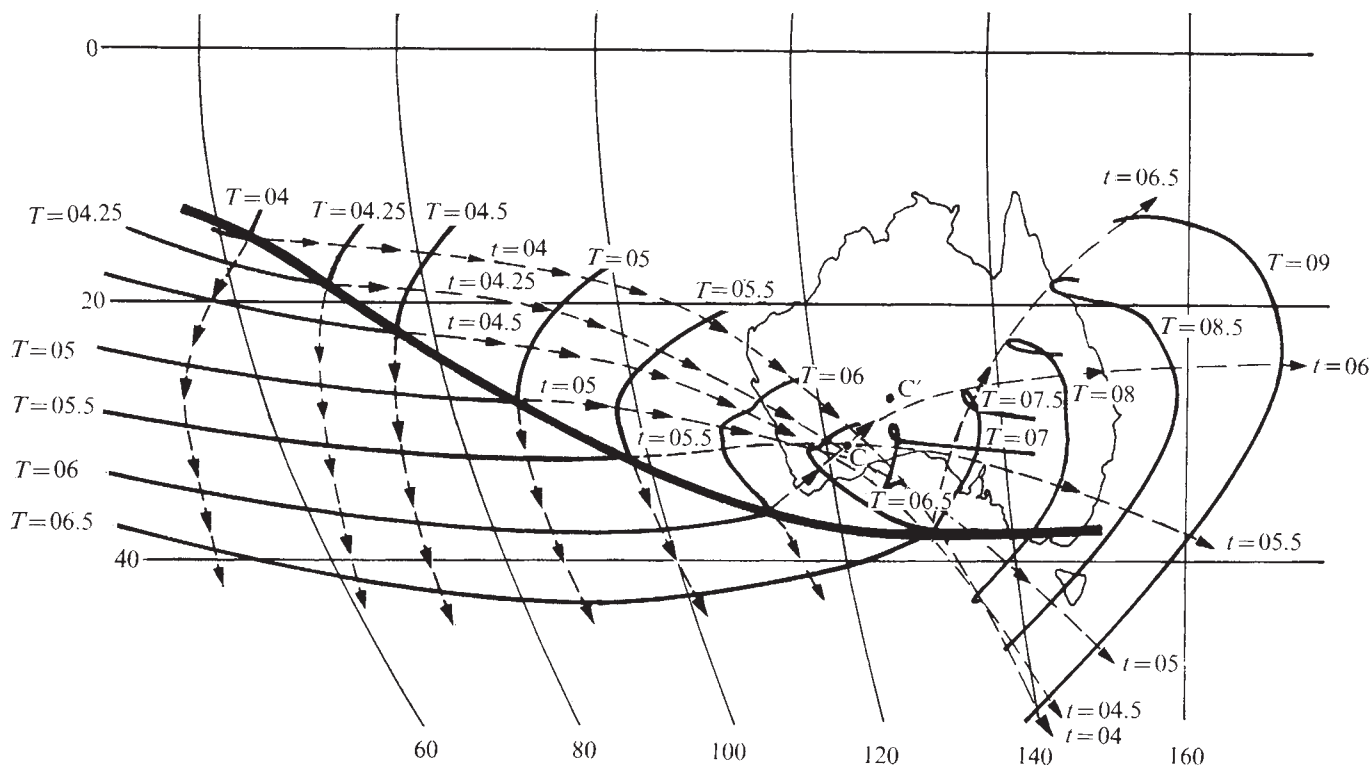


Fig. 1 Focusing of bow waves. The eclipse path is represented by a heavy continuous line. The wave fronts (continuous) are drawn for constant values of T . Ray paths (dashed) are shown for disturbances originating at times t . C_L is taken to be $10.3^\circ \text{ h}^{-1}$. When a value of 7.8° h^{-1} is assumed, the focus at C moves to C' .

The calculations were made for two values of C_L , the velocity of propagation of the bow wave, namely $10.3^\circ \text{ h}^{-1}$ (320 m s^{-1}) and 7.8° h^{-1} (290 m s^{-1}). Actual propagation velocities of long period internal gravity waves are likely to be between these values.

A well defined focus, near C in Fig. 1, appears in Western Australia where the southwards waves generated between approximately 0400 UT and 0600 UT tend to converge for $C_L = 10.3^\circ \text{ h}^{-1}$. C' in Fig. 1 indicates that focus for $C_L = 7.8^\circ$

The recording of ground level microbarograph and ionospheric disturbances in the focal region would be most interesting.

TOM BEER

Physics Department,
Western Australian Institute of Technology,
South Bentley 6102,
Australia

G. L. GOODWIN
G. J. HOBSON

School of Physics,
South Australian Institute of Technology,
Australia

Table 1 Approximate position and occurrence time of focus

C_L ($^\circ \text{ h}^{-1}$)	7.8	10.3
Latitude ($^\circ \text{S}$)	27	32
Longitude ($^\circ \text{E}$)	127	123
T (UT)	07.5	06.6

Received October 4; accepted October 15, 1976.

- Chimonas, G., and Hines, C. O., *J. geophys. Res.*, **75**, 875 (1970).
- Davis, M. J., and da Rosa, A. V., *Nature*, **226**, 1123 (1970).
- Arendt, P. R., *J. geophys. Res.*, **76**, 4695 (1971).
- Schödel, J. P., Klostermeyer, J., and Röttger, J., *Nature*, **245**, 87 (1973).
- Hunter, A. N., Holman, B. K., Feldgate, D. G., and Kelleher, R., *Nature*, **250**, 205 (1974).
- Beer, T., and May, A. N., *Nature*, **240**, 30 (1972).
- Frost, A. D., and Clark, R. R., *J. geophys. Res.*, **78**, 3995 (1973).
- Fiala, A. D., and Duncombe, J. S., *U.S. Naval Observatory Circular* 152 (1975).
- Chimonas, G., and Hines, C. O., *J. geophys. Res.*, **76**, 7003 (1971).
- Chimonas, G., *J. geophys. Res.*, **75**, 5545 (1970).

Argon fluorescent X rays in the Earth's atmosphere during solar flares

THE satellite Ariel V carries a proportional counter pointed along the spin axis. We report here the detection of a strong flux of X rays coming from the sunlit Earth during two large solar flares. These albedo X rays are proportional to the solar X-ray flux and consist of scattered X rays and a strong fluorescent line at 3 keV from atmospheric argon. There is also a weaker line at ~ 7 keV which is probably present in the incident solar spectrum.

The MSSL proportional counter (Experiment C) on Ariel V is a 92-cm² aperture, 85- μm thick Be window, Xe-filled counter. The field of view is circular with FWHM = 3.5° and is inclined 1.75° to the spin axis. This results in a field of view

h^{-1} . The approximate position and T for the foci are indicated in Table 1. C and C' identify a comparatively small region where enhanced bow wave amplitude should occur.

The focus is well within the penumbra region where the bow wave contains predominantly shorter wavelength components. Quasi-periods of ~ 20 min should therefore be observed^{2,9}, rather than longer periods of some 4 h which should predominate at great distances¹⁰.

Some aspects of the discussion are over-simplified. Firstly, the eclipse path is regarded as a curved line following the path of totality, so that disturbances generated within the penumbra are ignored. Secondly, the discussion considers only geometrical optics, whereas diffraction effects will produce a more diffuse focus than indicated in Fig. 1. A disturbance period of ~ 20 min corresponds to a wavelength of 350–400 km, which will be the order of the diffraction pattern diameter.

Finally, the bow wave is assumed to propagate at constant speed along great circles, and complications arising from the structure of the atmosphere are ignored.

(FOV) of 5.2° FWHM for the observations to be described. The experiment operates in one of two gain modes originally covering the energy range 1.5–20 keV.

Ariel V is in a near equatorial orbit with inclination 2.9° , apogee and perigee 543 and 501 km, and period 102 min. The satellite spins about its axis once every ~ 6 s.

During the flare of March 28, 1976, the experiment was in a low-gain multiscaler mode. The data recorded were total counts in the energy range 2.0–6.3 keV as a function of time in 64-s intervals.

During the flare of April 30, 1976, the experiment was in a spectral mode. The pulse height spectrum was recorded in 32 channels covering the energy range 1.5–9.5 keV and integrated over each orbit. Since the experiment operates only when the satellite is in sunlight, and is turned off when in the region of high charged particle flux over the South Atlantic, the average on-time is ~ 50 min. During this time the satellite views in sequence: a region of space containing the cosmic X-ray source of interest, the sunlit Earth, and the dark Earth. The sunlit Earth was a very strong X-ray source during the flare on April 30. The spectrum of the sunlit Earth was easily derived using the spectra recorded during the previous orbits (before the flare) as background.

For the flare on March 28, the spin axis was pointed at RA = 235° , dec. = $-1^\circ 6'$. No strong X-ray sources were in the FOV. The counting rate pointed at space was ~ 2 counts s^{-1} . This decreased to ~ 1 count s^{-1} when the dark Earth passed through the FOV and diffuse X rays from space were cut off. Just after the flare the rate increased to 5,000 counts s^{-1} when the sunlit Earth was viewed. Since the space rate remained at ~ 2 counts s^{-1} , there is no doubt that the sunlit Earth was the source of this signal.

The solar X-ray flux was monitored in two energy bands by NOAA ion chambers aboard the satellite GEOS 1 (ref. 1). These bands were 1–8 Å (1.5–12 keV) and 0.5–4 Å (3–24 keV). Ariel data can be normalised to the solar 1–8 Å flux or the 0.5–4 Å flux. The scattered flux seems to decay at about the same rate as the solar flux in either of these bands. We will use the 1–8 Å band because the ion chamber which produces these data has a window of about the same thickness as that of the Ariel detector. During the two flares which we observed, the solar flux in the 0.5–4 Å band was one tenth of that in the 1–8 Å band.

Figure 1 shows that the solar flux can be used to predict the X-ray strength of the sunlit Earth. When the solar flux is

Fig. 1 The solar X-ray flux in two energy ranges, (—, 0.5–4 Å; ---, 1–8 Å) recorded¹ during the flare of March 28. The right hand scale refers to the solar flux. The scale factor for each curve has been indicated. Note that the two curves have been normalised. The short heavy lines are the normalised counting rate of the Ariel detector. These rates are given on the left hand scale and the numbers (for example, 8,051) are orbit numbers. As expected, the albedo X rays follow the measured solar flux.

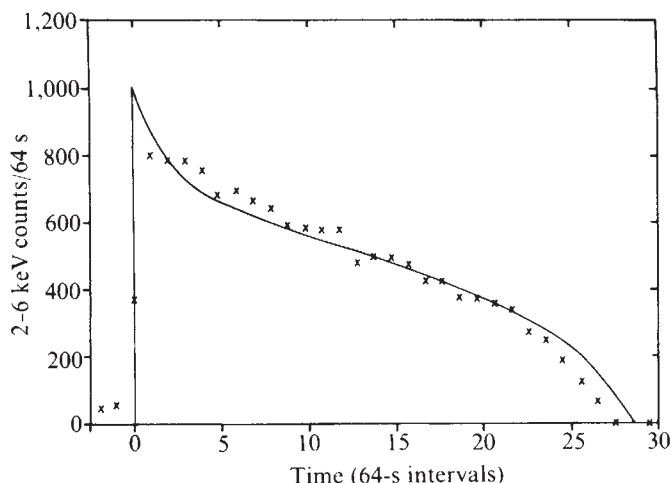
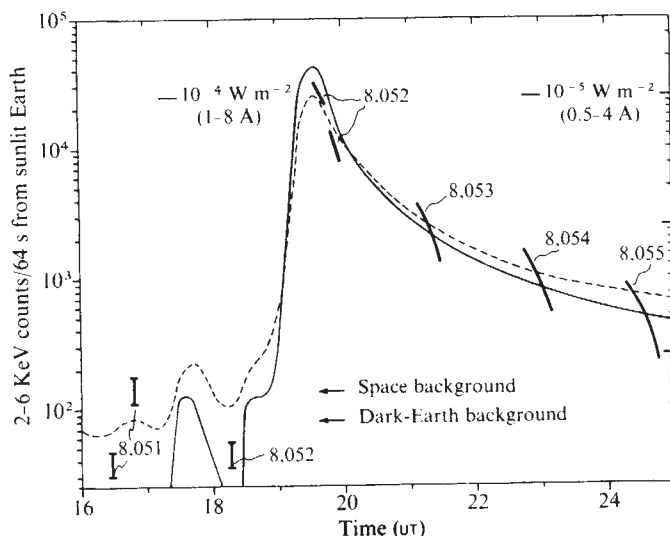


Fig. 2 The solid curve is the predicted dependence of scattered X-ray flux on scattering angle. It has been normalised to data taken during orbit 8,055 (x) to show that we understand the basic scattering geometry.

$5 \times 10^{-7} \text{ W m}^{-2}$ in the 1–8 Å band the scattered signal is expected to be about the same as the space background. Normally, when the Sun is not active, solar energy is $< 10^{-7} \text{ W m}^{-2}$ and the sunlit Earth is not a large source of background in our energy range. (This is not at all the case for softer X rays, particularly < 1 keV where fluorescent N and O lines are strong².)

Before deriving the scattered solar flux it is necessary to consider the geometry of our observation. Figure 1 shows that during each orbit the Ariel counting rate decreased much more rapidly with time than the solar X-ray flux. Since the satellite spin axis remains fixed in space, as the satellite circles the Earth it views different areas of the top of the atmosphere, and the angle that detected scattered X rays make with the terrestrial radius vector changes. The total thickness of atmosphere that these X rays must traverse, to travel from Sun to Earth to detector, also changes. Thus the detected scattered flux depends strongly on the position of the satellite in its orbit.

A simple calculation fits our data well. For these particular observations, the top of the atmosphere viewed by the detector was approximately planar, the X-ray absorption coefficient was almost the same for incident and scattered X-ray, there was only single scattering, and the cross-section did not vary much with angle (the scattering angle was always $> 90^\circ$). If the angle between the path of the incident X ray and the radius vector is ϕ , the angle between the path of the scattered X ray and the radius vector is θ , and the solar flux is f_0 , then the scattered flux at the satellite, f_s , is

$$f_s = \text{constant} \times f_0 \left\{ 1 + \frac{\cos \theta}{\cos \phi} \right\}^{-1}$$

This is shown in Fig. 2.

The agreement with our data is quite good considering that we have not smeared the prediction over the 5° detector FOV and that we have not included the time dependence of the solar flux.

The maximum flux in Fig. 2 corresponds to a time when the satellite views the sunlit limb of the Earth and the detector is looking parallel to the top at the atmosphere ($\theta = 90^\circ$). When the scattered flux drops rapidly to zero the detector is looking at the terminator ($\phi = 90^\circ$) and solar X rays are absorbed before they reach that part of the atmosphere viewed by the detector.

We note that scattering in our energy band must occur predominantly at altitudes between 70 and 90 km. At these altitudes and at sea level $\sim 1\%$ by volume of the atmosphere is argon.

We also note that Harries and Francey³ observed scattered solar X rays in the range 2–6 keV and showed that their rocket-borne detector background was consistent with this.

For the flare of April 30, spectra were recorded during orbits 8,555 and 8,556, which both showed a scattered solar continuum and a strong argon fluorescent line. We have analysed the data from orbit 8,555 which were recorded on April 30 from 2144 to 2203 UT. During this interval the solar flux averaged $2 \times 10^{-5} \text{ W m}^{-2}$ in the 1–8 Å band⁴.

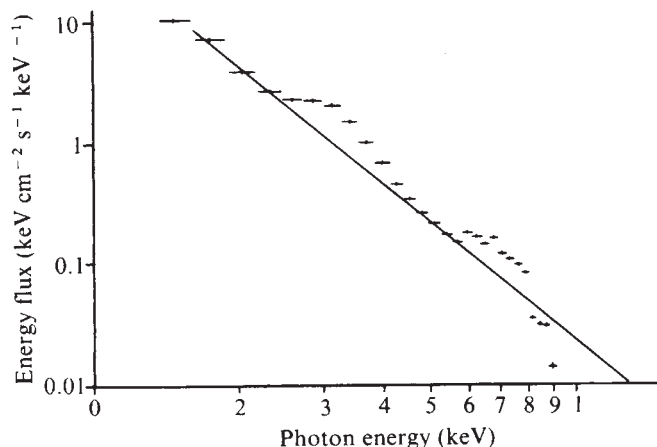
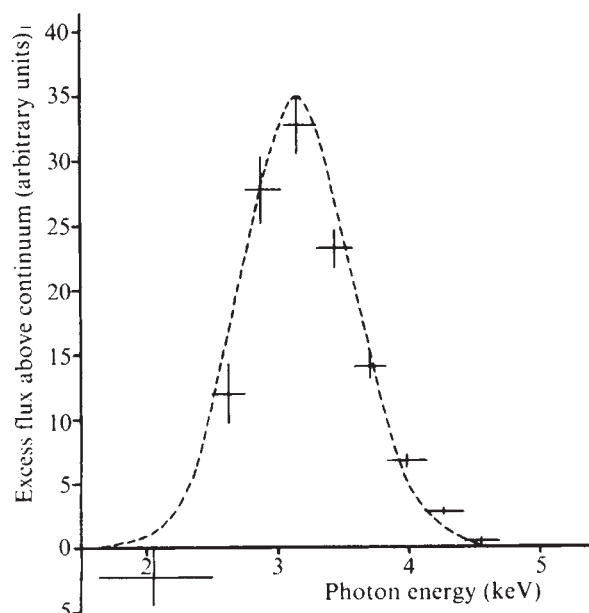


Fig. 3 X-ray energy scattered from the sunlit Earth measured during orbit 8,555. The argon fluorescent line and a scattered solar Fe XXV line(s) can be seen superimposed on the solid line which approximates the scattered solar continuum. Error bars show counting statistics only. Above 8 keV there is an additional uncertainty caused by background subtraction. The deviation of the last points from the line is not significant.

A continuum shown as a solid line in Fig. 3 was subtracted from the data to yield the line profile shown in Fig. 4. The energy and width of the line at ~3 keV are very close to the expected response of our detector to the argon line. The line is not exactly centred at the expected energy of 2.95 keV, because of the uncertainty in our detector calibration. The relative strength of the line and scattered continuum are as expected. This feature is without doubt the argon fluorescent line.

The solar spectrum during a flare consists of a continuum with strong lines due to Si XIII at 1.8 keV, Si XIV at 2.0 keV,

Fig. 4 The observed line shape compared to the expected detector response to a line at ~3 keV. Error bars are counting statistics only and do not include uncertainties in the continuum which has been subtracted.



Ca XIX at 3.9 keV and Fe XXV at 6.5 keV (ref. 5). The scattered Fe XXV feature is present in the spectrum illustrated in Fig. 3, but the other lines, if present, are hidden by the fluorescent A line at 3 keV.

We assume that the detector was looking at the Earth in the same direction as incident solar radiation ($\theta = \phi$), and also that the satellite was in a near-Earth orbit, so that the Earth can be considered as a planar source and the detector count rate is independent of altitude. The numbers following are calculated for a solar flux of $1.0 \times 10^{-4} \text{ W m}^{-2}$ in the 1–8 Å band, the maximum flux during each of the two flares we observed.

From the spectral data we derive 550 argon photons $\text{cm}^{-2} \text{ s}^{-1} \text{ ster}^{-1}$. This is approximately equal to the number of scattered photons $> 3.2 \text{ keV}$.

The total albedo energy from 1.5 to 9.0 keV (1.4–8 Å) is $1.5 \times 10^4 \text{ keV cm}^{-2} \text{ s}^{-1} \text{ ster}^{-1}$. This is equivalent to 1.5×10^{-3} of the incident solar energy re-emitted into 2π steradians. Eleven per cent of this albedo energy is in the argon line. The strength of the argon fluorescent line has been calculated by Aikin⁶ for quiet Sun conditions, but our observations do not agree—we get a factor of 100 less than Aikin.

For our detector, the rate at the peak of the flare caused by the X-ray albedo (1.5–9 keV) was 440 counts s^{-1} , 100 counts s^{-1} came from the A line and 7 counts s^{-1} from the scattered Fe line.

The X-ray flux from the Sun is normally $< 10^{-7} \text{ W m}^{-2}$ in the 1–8 Å band. Thus the normal background contribution of the sunlit Earth should be $< 0.4 \text{ counts s}^{-1}$. By way of comparison the dark-Earth background is 1 count s^{-1} and the background looking at a source-free region of space is 2 counts s^{-1} .

We thank the SRC and the USERDA for support, and Prof. R. L. F. Boyd, and the MSSL for hospitality. B. H. was on study leave from the University of Adelaide, Australia.

F. D. SEWARD
B. HORTON
G. POLLARD
P. W. SANFORD

Mullard Space Science Laboratory,
University College London,
Holmbury St. Mary, Dorking, Surrey, UK

Received July 26; accepted October 21, 1976.

- ¹ *Solar-Geophysical Data*, 380 Part I, 29, April 1976, U.S. Department of Commerce (Boulder, Colorado).
- ² Grader, R. J., Hill, R. W., and Seward, F. D., *J. geophys. Res.*, 73, 7149–7153 (1968).
- ³ Harries, J. R., and Francey, R. J., *Aust. J. Phys.*, 21, 715–723 (1968).
- ⁴ *Solar-Geophysical Data*, 381 Part I, 23, May 1976.
- ⁵ Doschek, G. A., *Space Sci. Rev.*, 13, 765–821 (1972).
- ⁶ Aikin, A. C., *Nature*, 227, 1334 (1970).

Penetration of interstellar dust into the Solar System

THE Sun moves through local interstellar matter with a velocity of $\sim 20 \text{ km s}^{-1}$ (refs 1–4). Bertaux and Blamont¹ have suggested that interstellar dust grains streaming into the Solar System are focused by the gravitational attraction of the Sun and that, as a consequence, the density of these dust grains is strongly peaked on a line extending from the Sun backwards in the downstream direction. Comparison of the predictions of this focusing model with direct observations of the dust⁵ (which do not show this large peak) leads Bertaux and Blamont to suggest that interstellar dust particles are underabundant in the Solar System by a factor of ~ 100 , compared with the usual picture of the density of interstellar grains. They suggest a number of possible explanations: first, the experiments are only sensitive to a fraction of the dust particles; second, radiation pressure or some other process eliminates the particles; third, the interstellar gas and dust near the Sun are abnormal. We point out here that a general effect not considered by Bertaux and Blamont offers a natural explanation of the observations. Grains, both in interplanetary and in interstellar space, will, in

general, carry a net electric charge, and the Lorentz force is strong enough to destroy gravitational focusing.

Several processes contribute to the charging of grains in space. The ejection of photoelectrons and the accretion of ions tends to charge the grains positively, while the accretion of electrons produces a negative charge. Since the cross section for the accretion of charged particles depends on the electrical potential of the grain⁶ the equilibrium charge state is given by a relation of the form

$$\sigma_e(V_0)f_e = \sigma_i(V_0)f_i + y\sigma_{ph}f_{ph} \quad (1)$$

where the σ are the cross sections of the grains for electrons, ions and energetic photons and the f are the fluxes of electrons, ions and energetic photons; y is the photoelectron yield (typically ~ 0.1 (ref. 7)) and V_0 is the electrical potential of the grain. Watson⁷ has computed the equilibrium electrical potential of grains in the interstellar medium to be positive, from ~ 0.1 V in cool, dense regions of interstellar space to ~ 3 V in hot tenuous regions. Grains impinging on the Solar System are thought to originate in a hot tenuous region⁴ and would thus be charged to ~ 3 V. Within the Solar System grains are subjected to large fluxes of electrons and ions from the solar wind and to energetic photons from the Sun. The photon flux is larger than the electron flux, so the grains are positively charged to a potential estimated at ~ 10 V (ref. 8). The sign of the potential is irrelevant to our argument.

A grain of radius a charged to a potential V_0 carries a net charge

$$q = aV_0/300 \text{ e.s.u.}$$

If the grains have radii⁴ of 2×10^{-6} cm and a nominal electrical potential of 5 V, then $q = ze|e| \simeq 3.3 \times 10^{-8}$ e.s.u. (corresponding to a deficit of ~ 70 electrons per grain).

The Lorentz force exerted by the solar wind magnetic field on a grain having velocity V_g is

$$\mathbf{F}_L = q \left[-\frac{\mathbf{V}_{sw} \times \mathbf{B}_\perp}{c} + \frac{\mathbf{V}_g \times \mathbf{B}}{c} \right] \quad (2)$$

where $\mathbf{B}_\perp$ is the component of the magnetic field perpendicular to the radial solar-wind velocity vector. The solar-wind velocity, V_{sw} , is ~ 400 km s⁻¹, so the initial velocity of the grain makes a negligible contribution to the Lorentz force. If we denote by $\mathbf{B}_\perp^0$ the transverse component of the magnetic field at the orbit of Earth and assume the field to have an Archimedian spiral structure⁹, then equation (2) can be written

$$|\mathbf{F}_L| = \frac{qV_{sw}|\mathbf{B}_\perp^0|}{c} \frac{\sin\theta}{r} \quad (3)$$

where r is expressed in astronomical units and θ is the solar colatitude angle. Then if $\mathbf{F}_G$ denotes the gravitational attraction of the grain by the Sun

$$\frac{|\mathbf{F}_L|}{|\mathbf{F}_G|} = \frac{(2.25 \times 10^{26})qV_{sw}B_\perp^0 r \sin\theta}{GM_\odot mc} \quad (4)$$

where as above $z = |q/e| \sim 70$. Estimating the radius of the solar wind cavity to be ~ 50 AU, and taking the average value of $\sin\theta$ to be ~ 0.5 , for the grains treated in the gravitational focusing model, $F_L/F_G \sim 10$ at the orbit of Earth, and $F_L/F_G \sim 500$ in the outer reaches of the solar wind. Thus it is clear that unless the estimates of the grain charge are grossly in error, the Lorentz force dominates the motion of all but the most massive grains throughout most of the Solar System; the Sun's gravitation is a minor perturbation to the motion of small, electrically charged grains in the Solar System.

The precise effect of the Lorentz force on the trajectories of grains depends on the geometry of the interplanetary magnetic field. There is evidence that the interplanetary field has a generally uniform large scale structure¹⁰. For our purposes, it is sufficient to compare the deflection of a grain produced by the Lorentz force to that produced by the Sun's gravity. A grain moving at an average velocity of 30 km s⁻¹ spends ~ 8 yr crossing to the centre of a 50-AU solar wind cavity. In that time the Sun's gravity deflects the grain's trajectory less than a radian, corresponding to a transverse velocity increment of ~ 10 km s⁻¹. Integrating the Lorentz force (equation 2) over a particle's trajectory yields a transverse velocity increment $|\Delta V|$ about equal to the smaller of $8.6 \times 10^3 z \ln(r_0) \text{ cm s}^{-1}$ or V_{sw} , where r_0 is the radius of the solar wind cavity in astronomical units. For $z \simeq 70$ and $r_0 \simeq 50$, $\Delta V \sim 400$ km s⁻¹. This corresponds to the particle being 'picked up' by the solar wind, gyrating about the magnetic field, and being convected out of the Solar System. The precise structure of the magnetic field or the details of the grain's motion are not relevant to our main point, which is that deflection of charged grains by the Lorentz force will obliterate the gravitational focusing effect so that one should not expect to see the predicted peak.

The reader can easily convince himself that the conclusion is not strongly dependent on the parameters used in this discussion. Interstellar grains (except those having a charge to mass ratio $\ll \sim 10^{-3}$ of that presumed here) will not undergo solar-gravitational focusing and will, in fact, be largely excluded from the Solar System by the sweeping action of the solar wind magnetic field.

We are grateful to N. J. Woolf for a helpful discussion. This work was supported in part by NASA.

E. H. LEVY

Department of Planetary Sciences and
Lunar and Planetary Laboratory

J. R. JOKIPPI

Department of Planetary Sciences and
Department of Astronomy,
University of Arizona,
Tucson, Arizona 85721

Received August 9; accepted October 7, 1976.

- ¹ Bertaux, J. L., and Blamont, J. E., *Astr. Astrophys.*, **11**, 200 (1971).
- ² Thomas, G. E., and Krassa, R. F., *Astr. Astrophys.*, **11**, 218 (1971).
- ³ Weller, C. S., and Meier, R. R., *Astrophys. J.*, **193**, 471 (1974).
- ⁴ Bertaux, J. L., and Blamont, J. E., *Nature*, **262**, 263 (1976).
- ⁵ Alvarez, J. M., *IAU Coll.* (Heidelberg, 1975).
- ⁶ Spitzer, L., Jr., *Astrophys. J.*, **93**, 369 (1941).
- ⁷ Watson, W. D., *Astrophys. J.*, **176**, 103 (1972).
- ⁸ Parker, E. N., *Astrophys. J.*, **139**, 951 (1964).
- ⁹ Parker, E. N., *Astrophys. J.*, **128**, 644 (1958).
- ¹⁰ Levy, E. H., *Nature*, **261**, 394 (1976).

Seiches in supergranules

THE recent report by Hill, Stebbins and Brown¹ of oscillations of an apparent solar radius has stirred a mild controversy². In particular, the failure of Grec and Fossat³ to detect Doppler variations with similar periods in solar spectral lines provides striking contrast with the results of Hill *et al.* Both sets of observations have been carefully performed and thoughtfully analysed and, if one accepts both sets of results at face value, the problem of reconciling the two must be confronted. Here we consider seiches in supergranules as a possible cause of the discrepancy.

Hill *et al.* in Arizona detected a discrete spectrum of oscillations in an effective diameter of the Sun, with periods decreasing from ~ 1 h. Apparent variations in this diameter can be caused by changes in the limb darkening function arising from temperature and density perturbations, but, if one assumes that the measures correspond to variations of the radius, one infers motions of the solar surface with velocities ~ 10 m s⁻¹. On the other hand Grec and Fossat in Nice have measured

the radial velocity of the solar material, averaged over the bulk of the solar surface, and find no motion with mean velocities $> 70 \text{ cm s}^{-1}$ on timescales $\sim 1 \text{ h}$ (though oscillations with a period of 5 min are clearly seen). If Hill's results arose from coherent oscillations of the solar surface it would be hard to explain the failure of the Nice group to see them, even if the Arizona results were partly a result of temperature variations.

The apparent discrepancy would be resolved if the variations detected by Hill *et al.* are the result of motions which are not coherent over the solar surface, so that they would be washed out in the Nice measurements. Indeed, the technique of Hill *et al.* would be particularly sensitive to motions with a coherence scale comparable with that over which they average.

Without going into the details of the apparatus of Hill *et al.*, we note that their slit length is $\sim 100''$, which corresponds to twice the supergranular scale of 30,000 km. Thus the results of Hill *et al.* represent an average over a few supergranules, whereas the Grec-Fossat measurements average over $\sim 1,000$. Appropriate motion in the supergranules could therefore explain the difference in results.

Supergranule motions are seen in the solar surface and look like convective cells, with upwelling in the centres and with horizontal outflow over most of the surfaces at speeds of $\sim 0.5 \text{ km s}^{-1}$ (refs 4, 5). These motions are distributed over the solar surface in a roughly polygonal network. At parts of the interface between the supergranules, vertical magnetic fields are observed with intensities of $\sim 1,800$ Gauss (ref. 6). These fields are concentrated in small spots which are located preferentially at the corners of the supergranules. Unfortunately, we have no direct knowledge of the field topology below the visible layers of the Sun. On theoretical grounds it is believed that these fields blend into a prevalent toroidal field well below the surface. Whether the observed flux ropes merge in relatively shallow layers or remain discrete over great depths is, however, not known.

Let us suppose the former possibility. Then the lower parts of the supergranules are partly separated by the magnetic field. Thus, we may consider the fluid in each supergranule to be loosely contained in a roughly fashioned, rather leaky cup. The cup walls would of course be flaccid, since the fields would probably be much weaker than the values observed in the small dots seen in the surface layers.

We propose that within these magnetic cups there occur seiches (slow sloshing motions). Such motion is well known to limnologists and is readily observed in a mug of beer held in an unsteady hand. The motion has little shear, and should therefore have low dissipation except in the very turbulent upper layer. At the speed suggested by the observations of Hill *et al.*, the supergranular seiches should be nearly incompressible. If we presume that the depth of a cup, h , is $\sim l$, the supergranular diameter, then the scale height near the bottom is $\sim h$; only in the upper layers do we face great inhomogeneity. We may therefore estimate the period P of the fundamental seiche mode using Merian's formula⁷

$$P = \frac{2l}{(gh)^{\frac{1}{2}}} = 11 \left(\frac{l}{h} \right)^{\frac{1}{2}} \text{ min}$$

where g is the solar surface gravity. Of course this is a very crude estimate, since a number of complicating factors have been ignored. In particular, the restraining effect of the magnetic field has probably been grossly exaggerated, and it is likely that the period should be considerably longer than this formula suggests. Nevertheless, Merian's formula gives some idea of the order of magnitude of the period if we know the value of l/h .

It has been suggested⁸ that supergranules are squat with $l/h \simeq 5$. The arguments for this are not compelling, and in fact we do not know why there should be such a distinct cell size in what appears to be a very turbulent layer. Moreover, we have to distinguish between the depth to which traces of the horizontal pattern persist and the depth we would attribute to a coherent

motion of the sort we are contemplating. The latter depth, which is h in our picture, is that at which the toroidal magnetic field becomes appreciable and is able to provide effective basins in which the supergranules may rest. If we suppose that the surface magnetic field is anchored in this toroidal field we can estimate this depth.

One might suppose that the depth dependence of the solar angular velocity Ω near the equator is such that the differential rotation maintains approximate neutrality according to Rayleigh's criterion. Then near the equator

$$\Omega \simeq \Omega_s \left(\frac{R}{r} \right)^2$$

where Ω_s is the surface angular velocity, R is the solar radius, and r is the radial co-ordinate. The value of Ω at the depth of interest is reflected by that of the sunspots and the magnetic network as seen at the surface. The difference between Ω and Ω_s is $\sim 5\%$ (ref. 4). This suggests depths of $\sim 2 \times 10^4 \text{ km}$. A similar argument has been advanced by Foukal and Jokopii⁹.

Thus we have estimates for l/h in the range 1–5 and Merian's formula suggests periods in the range of 10–30 min. The flexibility of the walls will raise these values, and periods $\sim 1 \text{ h}$ seem possible.

The way in which seiching of supergranules could affect the results of the Arizona group is complicated. Slight tilting in a suitable direction of a supergranule at the limb could modify the local limb darkening, to which the Arizona observations are very sensitive, but it seems premature to enter into such details in this very tentative model. We note that the kind of effect we allude to here gives a change in the apparent position of the limb. It may also give some change in local intensity, but if this is appreciable, the picture we propose would appear to be ruled out by recent work of Musman and Nye who have failed to detect brightness fluctuations on the solar disk with periods in the range of 15–90 min. To illustrate the kind of effect we envisage, it may be adequate to adopt the simplistic view that seiches would make their presence known through slight local elevations of the 'solar surface'.

If speeds in the seiche mode are $\sim 10 \text{ m s}^{-1}$, the picture is compatible with the observations of Hill *et al.* and also predicts that the observations made in Nice would provide negative results unless the sensitivity were increased to detect velocities significantly $< 1 \text{ m s}^{-1}$ or unless the area averaged over were appreciably reduced. Moreover, the energy in the motion is much less than that required if the observations are to be explained in terms of nearly pure global normal modes, since the seiches are probably not very deep. This is satisfying in view of the recent computations by Goldreich and Keeley¹⁰, which indicate that it might be difficult to excite such modes in the Sun by turbulent driving. As to the excitation of the seiches, various possibilities present themselves. For example, supergranules last $\sim 40 \text{ h}$, according to Simon and Worden, and seiches would probably be excited as part of the formation process. In addition, undulations of the deep toroidal magnetic field would provide rocking of the magnetic cups, and consequent excitation of the modes.

The idea of sloshing supergranules is based on assumptions about the subsurface field topology. Where the surface field emanates from the underlying toroidal field there should be significant deformations, perhaps ridges, in the toroidal field. But it may be that these structures lie too deep to confine the supergranules. Nevertheless, individual motions of supergranules might arise, though the existence of an adequate excitation mechanism is then less apparent. Among the possibilities for exploration is the collective interaction of supergranules, as also noted by N. O. Weiss. This might produce suitable surface undulations, but it is not clear what the period would be. This would also provide some degree of phase coherence. Surface waves travelling at the local sound or gravity wave speeds do, however, traverse a supergranule in $\sim 1 \text{ h}$ and long waves of this kind would have nodes at the super-

granule interstices where strong fields are found. This possibility becomes more interesting when one realises that solar surface waves may be unstable, as indicated by a recent study by L. Baker.

We believe, therefore, that there are sufficient grounds for suggesting that one should study motions of supergranules as individual entities. The amplitude of the motion needed to resolve the apparent conflict in the present observations suggests that the seiches would be detectable. Indeed, there are observed variations in the solar surface which indicate that such motions may actually exist¹¹.

We thank the SRC and the NSF for financial support. We are indebted to Dr N. O. Weiss for criticism and to D. J. Galloway for a discussion on solar hydromagnetics. Dr J. Beckers, Dr S. Musman and Dr G. W. Simon read the manuscript critically. Dr Simon also has informed us of his related work with S. P. Worden which aims to explain apparent long-period (2 h 40 min) oscillations^{12,13} as velocity fluctuations produced by the solar rotation of supergranules through the observed field of view.

Note added in proof: Hill¹⁴ has recently found evidence for phase coherence of the 45-min oscillation over 30 d and Kotov *et al.*¹⁵ have found phase coherence of the 2 h 40-min oscillation over 3 yr. Thus although supergranular seiching and the passage of supergranules across the field of view may contaminate the measurements, the above evidence would imply that the oscillation data cannot be attributed entirely to these phenomena.

D. O. GOUGH
J. E. PRINGLE
E. A. SPIEGEL

*Institute of Astronomy, and
Department of Applied Mathematics and Theoretical Physics,
University of Cambridge,
Cambridge, UK*

Received August 17; accepted October 21, 1976.

- ¹ Hill, H. A., Stebbins, R. T., and Brown, T. M., *Proc. V. Int. Conf. Atomic Masses and Fundamental Constants*, 622 (edit. by Sanders, J. H., and Wapstra, A. H.), (Plenum, New York, 1976).
- ² *Phys. Today*, 29, No. 5, 17 (1976).
- ³ Grec, G., and Fossat, E., *Astr. Astrophys.* (in the press).
- ⁴ Beckers, J., and Canfield, R. C., in *Physique des Mouvements dans les Atmosphères Stellaires* (edit. by Cayrel, R., and Steinberg M.), (CNRS, Paris 1976).
- ⁵ Weiss, N. O., in *Basic Mechanisms of Solar Activity* (edit. by Bumba, V., and Kleczek J.), (Reidel, Dordrecht, 1976).
- ⁶ Stenflo, J. O., *Solar Phys.*, 32, 41 (1973).
- ⁷ Proudman, J., *Dynamical Oceanography*, (Methuen and Co., London, 1953).
- ⁸ Bray, R. J., and Loughhead, R. E., *The Solar Granulation*, (Chapman & Hall, London, 1976).
- ⁹ Foukal, P., and Jokipii, J. R., *Astrophys. J., Lett.*, 199, L71 (1975).
- ¹⁰ Goldreich, P., and Keeley, D., *Astrophys. J.* (in the press).
- ¹¹ Zirin, H., *The Solar Atmosphere* (Blaisdell, Waltham, 1966).
- ¹² Severny, A. B., Kotov, V. A., and Tsap, T. T., *Nature*, 259, 87 (1976).
- ¹³ Brookes, J. R., Isaak, G. R., and van der Raay, H. B., *Nature*, 259, 92 (1976).
- ¹⁴ Hill, H. A., *Trans. int. astr. Union* (in the press).
- ¹⁵ Kotov, V. A., Severny, A. B., and Tsap, T. T., *Trans. int. astr. Union* (in the press).

Liquid immiscibility in K_2O -FeO- Al_2O_3 - SiO_2

DIFFERENTIATION in silicate magmas is usually considered to occur through processes of fractional crystallisation. The possibility that the formation of a second silicate liquid, immiscible with the original silicate magma, may take place has been thought unlikely¹. But in lunar rocks textures were found indicating late-stage formation of a K_2O -rich silicate liquid², and serious consideration is being given to the possible importance of silicate liquid immiscibility³. Current investigations on the role of silicate liquid immiscibility were started with the work of refs 4 and 5. Because of the importance of the two-liquid field in the current developments we have re-investigated the K_2O -FeO- Al_2O_3 - SiO_2 system. Electron scanning microscopy combined with electron microprobe analyses allowed us to delineate the extent of the two-liquid field as well as to determine the compositions of the two coexisting liquids.

In the previous study, the oxygen fugacity was maintained on the Fe-FeO buffer⁶. Our investigation was made at a slightly higher oxygen fugacity, using the Mo-MoO₂

buffer, which lies within the FeO stability field⁶. Runs were made at temperatures between 1,100 and 1,700 °C *in vacuo*. The samples were kept in molybdenum capsules.

The most interesting result of our study concerns the compositional range of the two-liquid field. It extends from the binary join FeO-SiO₂ to the stability field of leucite, (Fig. 1) and incorporates the two smaller separated fields found previously. This result is based on more than 150 runs at various temperatures. Runs with initial composition in between the two miscibility gaps found previously also produced two liquids. As an example two runs at 1,550 °C are shown in Fig. 1. Electron microprobe data show that the liquids are homogeneous, indicating that equilibrium was obtained. The co-existing liquids can be characterised as a FeO-rich liquid with a K_2O/Al_2O_3 ratio of ~ 0.6 and a SiO₂-rich liquid with a K_2O/Al_2O_3 ratio of ~ 1.

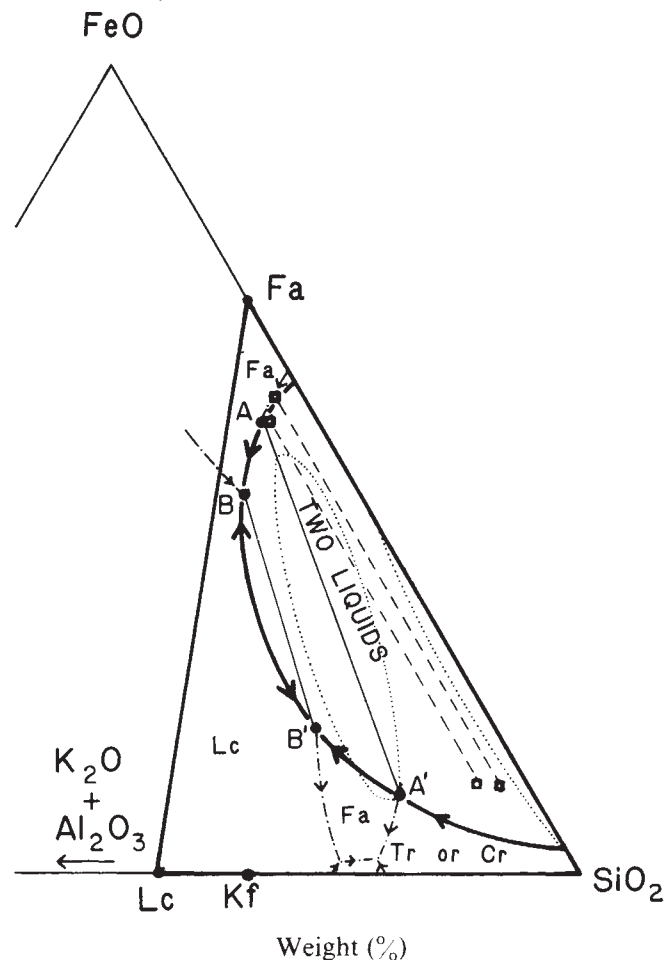


Fig. 1 Phase relations in the system K_2O -FeO- Al_2O_3 - SiO_2 *in vacuo*, projected on the plane leucite-fayalite-SiO₂. Oxygen fugacity buffered by Mo-MoO₂. Fa = fayalite, Lc = leucite, Tr = tridymite, Cr = cristoballite, Kf = K-feldspar. Dotted lines indicate the two-liquid fields found previously^{4,5}. □, Connected by — — —, examples of liquid pairs at 1,550 °C. At A-A' (1,150 °C) the assemblage fayalite + tridymite + 2 liquids is present. B-B' is the minimum temperature of the two-liquid field (1,130 °C).

The run products show that the two liquids form droplets within each other, which has been considered as the classical proof for liquid immiscibility¹. Figure 2a shows a typical example of textural features which were found in runs made at temperatures < 1,250 °C and > 1,400 °C; the size of the droplets varies from 3 mm to submicroscopic. In runs at intermediate temperatures separation of the two melts occurs on a submicroscopic scale only. Examples of this type of separation are shown in a series of scanning electron micrographs, see Fig. 2. If the etched glass surface did not show a texture

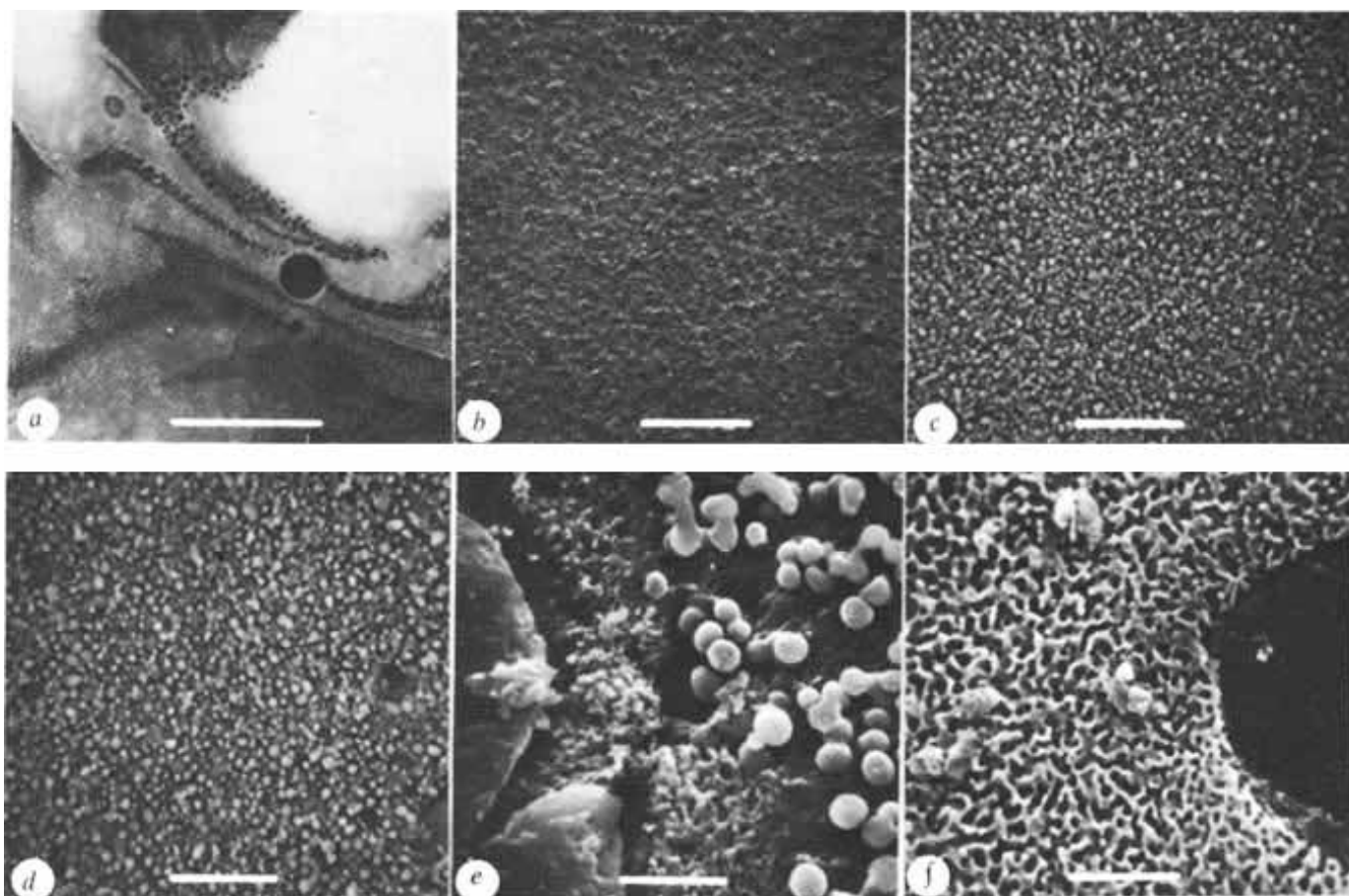


Fig. 2 Photographs of run products. Initial compositions lie roughly on the 30% FeO isopleth. *a*, Thin section of a typical run at low temperature (1,150 °C) in the middle of the two liquid field. The bar is 200 μ m long. Black spheres of a FeO-rich liquid in a matrix of colourless SiO₂-rich liquid. Note the variation in size of the spheres; grey cloudy areas (upper left) indicate submicroscopic scale separation. Pictures *b-f* are scanning electron micrographs of etched surfaces of run products. The etching agent was HF which preferentially removed the SiO₂-rich liquid. The bar is again 2- μ m long. *b*, Sample from one liquid field. Run temperature 1,300 °C (normal image). *c*, Sample from two-liquid field, just inside the two-liquid/one-liquid phase boundary. Run temperature 1,300 °C. White droplets are the SiO₂-rich glass (inverted image). *d*, Sample from the middle of the two-liquid field. Run temperature 1,300 °C. Size of the droplets is larger than in *c* (inverted image). *e*, Sample from the two-liquid field, close to the FeO-SiO₂ join. Run temperature 1,300 °C. Spheres are the FeO-rich liquid. On the left hand is a tridymite crystal (normal image). *f*, Sample from the leucite + two liquid field. Run temperature 1,200 °C. Black spherical cavities indicate the SiO₂-rich glass removed by etching. On the right side is a leucite crystal (normal image).

indicating unmixing (Fig. 2*b*) the liquid was considered to be homogeneous. If droplets of one liquid were found in a matrix of another, clearly two liquids were present, and the run was considered to lie within the two-liquid field (Fig. 2*c-e*). These textures are typical for a spinodal phase separation mechanism⁷. The size of the droplets increases toward the FeO-SiO₂ join. Close to this join tridymite or cristoballite was found together with the two liquids (Fig. 2*e*). An example of leucite plus two liquids is shown in Fig. 2*f*.

The phase relations of the liquidus surface are shown in Fig. 1. The two-liquid field intersects the liquidus surface at 1,695 °C on the FeO-SiO₂ join. It extends into the system and reaches a minimum at 1,130 °C (B-B') where FeO-rich liquid reacts to form fayalite + leucite + SiO₂-rich liquid. Thus, any Fe₂SiO₄-rich liquid must produce SiO₂-rich liquids during the course of crystallisation, without the formation of liquids of intermediate compositions. An important reaction occurs at 1,150 °C (A-A'). At this temperature tridymite reacts with the FeO-rich liquid to form fayalite plus SiO₂-rich liquid. Fayalite coexists with the SiO₂-rich liquid down to the eutectic.

The geological importance of these new data is that at least for dry magmas an FeO-rich basalt must yield an immiscible granitic liquid during the later stages of

crystallisation. Liquids of intermediate composition are not formed in this process. The immiscibility textures in lunar rocks² are an example of this. On Earth, excellent immiscibility textures were found in Archean variolitic pillow lavas near Noranda (Quebec, Canada) by Gelinis *et al.*⁸. In conclusion, the suggestion that the granophyres found in close association with ferro-gabbroic rocks may be produced by a process of liquid immiscibility⁹, appears to be valid.

WIEKERT VISSER
AUGUST F. KOSTER VAN GROOS

Department of Geological Sciences,
University of Illinois, Chicago,
Chicago, Illinois 60680

Received August 26; accepted October 19, 1976.

- 1 Carmichael, I. S. E., Turner, F. J., and Verhoogen, J., *Igneous Petrology* (McGraw-Hill, 1974).
- 2 Roedder, E., and Weiblen, P. W., *Science*, **167**, 641-644 (1970).
- 3 Watson, E. B., *Contr. Miner. Petrol.*, **56**, 119-134 (1976).
- 4 Greig, J. W., *Am. J. Sci.*, 5th series, **13** (1927).
- 5 Roedder, E., *Am. Miner.*, **36**, 282-286 (1951).
- 6 JANAF thermochemical tables. Dow Chemical Company (1965).
- 7 Cahn, J. W., *J. chem. Phys.*, **42**, 1, 93-99 (1965).
- 8 Gelinis, L., Brooks, C., and Trzcinski, W. E., Jr., *Can. J. Earth Sci.*, **13**, 210-230 (1976).
- 9 Irvine, T. N., *Geochim. cosmochim. Acta*, **39**, 991-1020 (1975).

Phosgene in the ambient air

IN recent years the atmosphere has become contaminated with a large number of halogenated compounds. Whereas inert fluorocarbons are suspect as precursors of stratospheric ozone-destroying chlorine atoms, other halocarbons, such as vinyl chloride, chloroform and trichloroethylene, have been found to be carcinogens^{1,2}. We present here the first measurements of ambient phosgene (COCl_2), and demonstrate that chloroethylenes (primarily C_2Cl_4 and C_2HCl_3), which are emitted worldwide in extremely large quantities (1.5×10^6 t in 1975), photo-decompose to form highly toxic species such as phosgene and chloroacetylchlorides. A consideration of the sources, the distribution, and the fate of these species suggests that a significant environmental impact is possible. The results presented here are based on laboratory and field studies conducted within California in 1976.

Phosgene is manufactured primarily for captive use, and its production in the United States in 1975 was in excess of 0.4×10^6 t. It is a highly toxic chemical, although very little is known about the health hazards associated with long term exposure to low concentrations of the gas³.

A special purpose electron-capture (EC) gas chromatograph (GC) equipped with two EC detectors in series was used for ambient phosgene analysis (Fig. 1). Calibrations were performed using a permeation tube and further checked in the field using the absolute pulse flow coulometric (PFC) method developed by Singh *et al.*⁴.

The phosgene was identified by comparing three independent properties, namely, GC retention data, the EC ionisation efficiency (Fig. 1), and phosgene destruction rates on glass surfaces with standard phosgene mixtures. The possibility of interference from other trace atmospheric halogenated species (such as CCl_3F , CCl_2F_2 , $\text{CCl}_2\text{FCClF}_2$, $\text{CClF}_2\text{CClF}_2$, CHCl_2F , CHClF_2 , CH_3Cl , CH_3I , CH_3Br , CH_2Cl_2 , CHCl_3 , CH_3CCl_3 , CCl_4 , C_2HCl_3 , C_2Cl_4 , $\text{CH}_2\text{BrCH}_2\text{Br}$, and SF_6) was eliminated by retention data measurements. The high ionisation efficiency of phosgene further precludes any possible interference, since only a few known species (such as CCl_4 , SF_6) are as good absorbers of electrons. An Ascarite trap in front of the GC column was able to remove phosgene as expected. A GC-MS confirmatory test was not possible because of the high liquid phase loading of the GC column and the unavailability of other acceptable column packings.

Field monitoring was conducted on a 24 h-a-day basis at four Californian sites (urban and non-urban) with the help of a mobile environmental laboratory. Table 1 provides the results of phosgene concentrations measured at these four sites, together with the concentrations of C_2Cl_4 and C_2HCl_3 , its suspected precursors. The range of phosgene concentrations

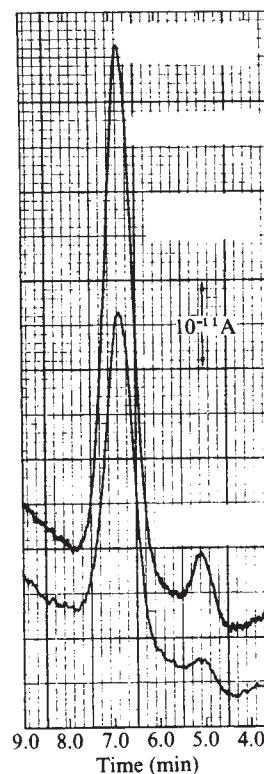


Fig. 1 Dual EC-GC chromatogram showing ambient phosgene elution for a 13.0-ml sample from Menlo Park. The separation was accomplished on a dual EC-GC, equipped with an aluminium column ($5' \times 0.25''$) packed with 30% didecyl phthalate on 100/120 mesh acid-washed Chromosorb P. This GC column was preconditioned with twenty, 5-ml injections of a 5 p.p.m. (10^{-6} v/v) mixture of phosgene and trichloroacetyl chloride in air. Both the GC column and the dual EC detector unit were maintained at 22°C . Ultrapure nitrogen (carrier gas) was passed through a sequence of traps containing charcoal, anhydrous calcium sulphate, and molecular sieve before entering the GC at a constant flow rate of 90 ml min^{-1} . All-glass syringes were used for sample injection, and the sample holdup time was kept < 15 s. The large peak is for fluorocarbon 11, retention time 6.9 min, p (ionisation efficiency) = 0.40; the small one is for phosgene, retention time 5.1 min, $p = 0.65$. The top curve is for ECD1, the bottom for ECD2, and the ionisation efficiency is given by $p = 1 - (\text{ECD2 signal/ECD1 signal})$.

was 13 to 61 p.p.b. (10^{-12} v/v) with a mean value of 21.6 p.p.b. at the remote site 3 which is $\sim 2/3$ the mean value of 31.7 p.p.b. in urban Los Angeles. This factor of 1.5 for the phosgene gradient between an urban and a remote site can be compared with a factor of 20 for its precursors. This primarily arises

Table 1 Average phosgene and precursor levels in California

Site no.	Location	Nature of site	Monitoring* period (1976)	Phosgene	Concentrations (10^{-12} v/v)	
					C_2Cl_4	C_2HCl_3
1	Lat. $34^\circ 04'$ Long. $118^\circ 11'$	Urban (Los Angeles)	28/4-4/5	31.7† (8.3)‡ Max. 61.1§	673.3 (496.8)	310.8 (301.6)
2	Lat. $33^\circ 48'$ Long. $116^\circ 33'$	Downwind of urban Los Angeles (Palm Springs)	5/5-11/5	29.3 (6.2) Max. 44.4	278.1 (232.7)	39.7 (83.6)
3	Lat. $37^\circ 39'$ Long. $119^\circ 40'$	Remote—High altitude (Badger Pass— elevation 7,800 feet)	12/5-17/5	21.6 (5.1) Max. 28.8	30.7 (10.5)	15.6 (2.5)
4	Lat. $37^\circ 24'$ Long. $112^\circ 12'$	Urban-suburban (Menlo Park)	23/5-27/5	30.3 (3.2) Max. 38.8	201.9 (413.9)	113.5 (528.5)

*All monitoring was conducted on a 24-h basis.

†The quantity before the parentheses is the mean of the entire data base.

‡The quantity in parentheses is the s.d.

§Maximum phosgene concentration.

because the precursors come from urban sources and are well dispersed by the time a significant decomposition to phosgene takes place.

The three readily identifiable sources of phosgene in air are: (1) direct emissions of phosgene during manufacture, handling, and use; (2) thermal decomposition of chlorinated hydrocarbons, and (3) photo-oxidation of chloroethylenes in the air. There are indications that the first two sources can result in a significant indoor hazard, but their contribution to the ambient budget is minimal. The photo-oxidation of chloroethylenes (C_2Cl_4 and C_2HCl_3) seems to be the most probable source of phosgene. Based on estimates from most recent laboratory studies⁵, present C_2Cl_4 and C_2HCl_3 emissions could result in the formation of $\sim 0.3 \times 10^6$ t of phosgene yr^{-1} . Because these solvents are typically emitted in urban areas and are relatively reactive (the tropospheric half lives of C_2HCl_3 and C_2Cl_4 are < 2 and 4 d, respectively), high concentrations of phosgene could be encountered during adverse meteorological conditions in and around urban centres.

The atmospheric sinks of phosgene are a matter of considerable uncertainty. The known absorption cross section of phosgene, and smog-chamber studies (where phosgene was found to be stable for 15 h under simulated tropospheric irradiations in the presence of 10,000 p.p.m. of water vapour) suggest negligible tropospheric loss through photolysis and gas-phase hydrolysis^{4,5}. The two important sinks of phosgene are heterogeneous decomposition and slow liquid-phase hydrolysis. The first sink is confirmed by the fact that phosgene at low concentrations was destroyed in contact with most surfaces within a few hours, and additionally, could not be measured in indoor spaces. During one day of field experiments, heavy overnight rain caused a 20% reduction in average phosgene concentrations. Slow dissolution in the ocean followed by hydrolysis is probably a significant sink of ambient phosgene. Other gas-phase reactions involving O and OH radicals are known to be very slow. It seems clear that phosgene is only removed slowly from the atmosphere.

Laboratory studies also suggest that the highly toxic di- and trichloroacetyl chlorides that are also formed from the photo-oxidation of C_2HCl_3 and C_2Cl_4 , respectively, would together be present at concentrations of about four times that of phosgene⁵. Methods for the measurement of chloroacetyl chlorides in the 10^{-1} – $1,000$ p.p.b. (10^{-12} v/v) range are not yet available. Secondary reactions of phosgene and chloroacetyl chlorides with ambient hydrocarbons and the heterogeneous decomposition of chloroacetyl chlorides to form chloro-methanes have already been suggested⁶. It is also possible that cigarette smokers may be exposed to an additional dose of phosgene, especially in urban locations, since chloroethylenes are thermally decomposed to phosgene, and are often present at concentrations of up to 200 times the ambient phosgene concentrations.

Because of the high toxicity of phosgene and chloroacetyl chlorides, and the suggested carcinogenicity of C_2HCl_3 and its structural similarity to C_2Cl_4 , the continuous release of these chloroethylenes in such large quantities to the atmosphere may be undesirable.

This project has been financed in part with federal funds from the EPA.

HANWANT BIR SINGH

Stanford Research Institute,
Menlo Park, California 94025

Received July 19; accepted October 7, 1976.

- ¹ Molina, M. J., and Rowland, F. S., *Nature*, **249**, 810–812 (1974).
- ² Preliminary Assessment of Suspected Carcinogens in Drinking Water (US Environmental Protection Agency, Washington, DC, 1975).
- ³ Occupational Exposure to Phosgene (NIOSH, HEW Publication No. (NIOSH) 76-137 (1976).
- ⁴ Singh, H. B., Lillian, D., and Appleby, A., *Analyt. Chem.*, **47**, 860–864 (1975).
- ⁵ Gay, B. W., Hanst, P. L., Bufalini, J. J., and Noonan, R. C., *Environ. Sci. Tech.*, **10**, 58–67 (1976).
- ⁶ Singh, H. B., Fowler, D. P., and Peyton, T. O., *Science*, **192**, 1231–1234 (1976).

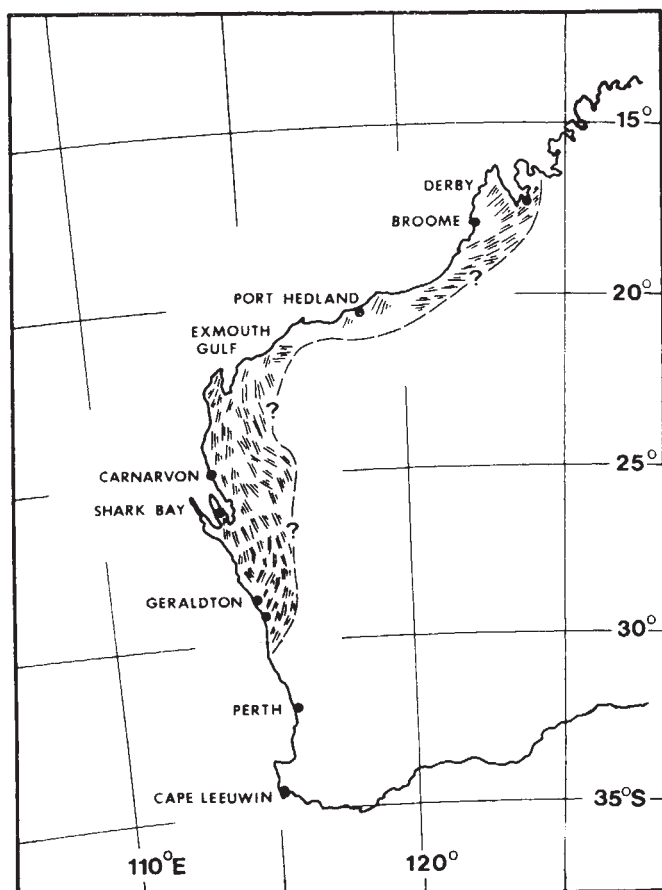
Widespread late Quaternary aridity in Western Australia

UNTIL recently the only conclusive evidence of extended arid conditions during the late Wisconsin over the western part of the Australian continent, was largely provided by the submerged dunes of the Fitzroy estuary^{1,2}. This evidence has been used to support the concept of global tropical aridity during the late Quaternary³. From a study of the late Quaternary sediments of the coastal areas of Western Australia, however, it has become apparent that stable dune fields and sand sheets of late Quaternary age are found throughout this region (Fig. 1).

It is evident from electron microscope studies of quartz grain surface textures, and from the grain-size distribution and mineralogy of the sands, as well as from the field occurrence and form of the sand deposits, that these deposits must be aeolian in origin, and derived from inland sources. In the Fitzroy estuary Holocene marine sediments overlie dunes, and give these dunes a minimum age of 7,400 b.p. (ref. 2). Linear dunes in the Exmouth Gulf have been truncated by the same transgression. Further south, a minimum age is more difficult to establish. In the Geraldton area, an inset valley fill dated at $3,145 \pm 145$ b.p., consisting largely of reworked aeolian sands indicates that arid conditions, in that area, must have ceased by that time.

A likely maximum age for the aeolian deposits can be established from the age of red alluvial sediments from which some aeolian material was deflated. In the Geraldton region the red alluvial fill overlies deposits of the Sangamon high sea level of $\sim 120 \times 10^3$ b.p. (ref. 4). Carbonaceous

Fig. 1 The recognised extent of wind-blown sand deposits in the coastal areas of Western Australia.



material from the red alluvial fill gave a ^{14}C age of $> 37 \times 10^3$ yr. The considerable thickness of the red alluvial sediments in coastal sections demonstrates that they were deposited at a time when the sea level was considerably lower than at present. An early Wisconsin age seems plausible for the red alluvium, and hence it follows that this is the maximum age of the aeolian sands.

The stratigraphy of the deposits gives no additional evidence of the precise age of the wind-blown sands. Pedocalcic palaeosols from the Timor Shelf, dated at 17,000 b.p. (ref. 5), and lunette deposits in the South-west of Western Australia, with a corresponding age of $\sim 17,000$ b.p. (ref. 6), indicate, however, that at that time arid conditions prevailed both in the North and South of the state. This provides a possible age for the aeolian sands. More important than this is the fact that the combined evidence suggests the possibility that most of the western part of the Australian continent may have been intensely arid at that time.

Although the climatic conditions necessary to bring about widespread and intense aridity over most of Western Australia would require a fundamental alteration of the present climatic regime, the actual mechanisms which can bring about such conditions require only relatively minor changes in the present heat balance and circulation pattern of the continent. The present climate of Western Australia is dominated by the subsiding air of the sub-tropical high pressure belts⁷. The seasonal displacement of this belt brings about the two distinct precipitation regimes: summer rainfall in the North, through the incursion of moist tropical air; winter rainfall in the South, from mid-latitude depressions.

The travelling anticyclonic cells within the sub-tropical high pressure belt control both precipitation regimes. In winter this is especially so. In these months the anticyclonic cells become intense, enhanced by low surface temperatures, and move slowly⁸. In these conditions the anticyclonic cells can dominate much of the continent, and in Western Australia limit rainfall to the southern shores. In some instances a large anticyclonic cell may produce a blocking effect, deflecting mid-latitude fronts approaching the West Australian coast southwards, and restricting precipitation to the south-western corner.

Even during the summer months, the anticyclonic cells, which are now situated much further South, have a profound influence on the summer precipitation regime of the northern areas. Often, when an intense surface anticyclonic cell is present in the Australian Bight, stable conditions prevail in the lower troposphere over northern Western Australia. This stability occurs in spite of the presence of a shallow hot surface region low over the Pilbara Region. Only when the anticyclonic cell moves eastwards, or decays, will the stable conditions over the northern areas weaken, resulting in an increased likelihood of rainfall from monsoonal depressions and disturbances^{9,10}.

It is evident from the present climatic situation over the Australian continent that an increase in anticyclonicity would directly result in widespread aridity over most of the western portion of the continent. The lower global temperatures which prevailed at the time of the last glacial maximum would have been sufficient to bring about an increase in anticyclonicity. With a lowering of the July temperature by $\sim 5^\circ\text{C}$ (refs. 11, 12), the winter anticyclone would have become much more stable, and the frequency of breakdown of anticyclonic cells would have been considerably lower. As a result winter rainfall would have been confined to the extreme south-west with only occasional widespread rains over the southern half of the state.

During the summer months anticyclonic conditions would have been weakened but this may have been only to such

a degree that the mean synoptic conditions would have been similar to the present-day late spring or early autumn. In addition, because of a more restricted seasonal migration of the inter-tropical convergence zone, the summer anticyclonic cells would have been located further north than at present. These factors, combined with weaker Trade winds, would have resulted in extremely stable conditions, curtailing the amount and frequency of summer rainfall over the northern areas.

An additional factor which must be considered is that cooler sea surface temperatures may have greatly reduced the significance of the offshore waters of northern Australia as a tropical cyclogenetic area¹³. Tropical cyclones contribute a considerable proportion of the present total precipitation of the northern areas of the state (for example, at Port Hedland this accounts for 50% of the mean annual precipitation). A decrease in the frequency of cyclones, whether through reduced cyclogenesis or a more westerly sea path because of stable conditions prevailing over the continent, would drastically alter the precipitation amounts in the northern half of the state.

The circulation changes necessary to result in aridity prevailing over most of the western parts of the Australian continent are physically plausible from what is presently known of global climatic conditions at the last glacial maximum. With the changes in the circulation patterns that have been proposed to explain the geomorphological evidence, it is difficult to envisage that arid conditions could have prevailed in the tropics without intense aridity further south. This reconstruction also implies that arid conditions prevailed through much of the glacial periods, becoming especially pronounced at times of glacial maxima. Even today Western Australia is very susceptible to the onset of arid conditions, a present drop of 120 mm in the total annual rainfall would extend the present margins of aridity by some $473 \times 10^3 \text{ km}^2$ (ref. 14). It follows that the envisaged circulation conditions prevailing at the last glacial maximum would have had their most obvious manifestations in Western Australia. While this is only to be expected, the increase in anticyclonicity would have had an effect on the whole continent, to such an extent that these conditions are likely to have been the determinant of Australian climatic conditions through the glacial periods.

We thank Dr J. Gentilli for his help. Mr G. Kendricks gave us considerable advice, and Dr J. M. Bowler let us use, as yet, unpublished ^{14}C dates.

KARL-HEINZ WYRWOLL

Department of Geography,
University of Western Australia,
Nedlands, Western Australia 6009

DEREK MILTON

Department of Geography,
University of Manitoba,
Winnipeg, Manitoba R3T 2N2, Canada

Received August 11; accepted October 10, 1976.

- 1 Fairbridge, R. W., in *Problems in Palaeoclimatology* (edit. by Nairn, A.), 356 (Interscience, New York, 1964).
- 2 Jennings, J. N., *Catena* (in the press).
- 3 Williams, M. A. J., *Nature*, **253**, 617–618 (1975).
- 4 Veeh, H. M., *J. geophys. Res.*, **71**, 3379–3386 (1966).
- 5 Van Andell, T. H., Heath, G. R., Moore, T. C., and McGeary, D. F. R., *Am. J. Sci.*, **256**, 737–758 (1967).
- 6 Bowler, J. M., *Earth Sci. Rev.* (in the press).
- 7 Gentilli, J., in *World Survey of Climatology*, **13** (edit. by Landsberg, K.), 35–211 (Elsevier, Amsterdam, 1971).
- 8 Kidson, E., *Aust. Bur. Met. Bull.*, **17** (1925).
- 9 Falls, R., *Proc. Symp. Trop. Met.*, **E.V.8** (Am. Met. Soc./W.M.O., Honolulu, 1970).
- 10 Rutherford, G. T., and Hannan, F. T., *Proc. Tropical Cyclone Symp.*, 379–390 (Bur. Met. Aust., Melbourne, 1956).
- 11 Gates, W. L., *Science*, **191**, 1138–1144 (1976).
- 12 Climap members, *Science*, **191**, 1131–1136 (1976).
- 13 Webster, P. J., and Streten, N. A., in *Bridge and Barrier: The Natural and Cultural History of Torres Strait* (edit. by Walker, D.) 39, (Research School of Pacific Studies, Pub. B6/3, A.N.U. Canberra, 1972).
- 14 Gentilli, J., *West. Aust. Nat.*, **2**(8), 175–184 (1951).

Evolution of ocean climate and the record of planktonic foraminifera

THE planktonic foraminiferal fossil record reveals two major evolutionary radiations, one in the Palaeogene and another in the Neogene. Preceding each radiation, at the beginning and middle of the Cainozoic, there were severe reductions in faunal diversity. Both reductions involved an elimination of tropical and sub-tropical forms and the radiations involved their restoration¹.

During the late Cretaceous and the Palaeogene (until about the Middle Eocene) faunas were diverse and well differentiated. Douglas² has shown that the late Cretaceous faunas, like modern ones, can be divided into cold- and warm-water groups (Boreal and Tethyan) and recently Szczuchura and Pozaryska³ have shown that the same is true for Palaeogene faunas. Both late Cretaceous and Palaeogene faunas, however, indicate a much greater expanse of warm water than exists today⁴.

In contrast, Danian and Oligocene faunas universally have a 'cold water' aspect. They are completely lacking in warm water morphotypes and diversities are consistently low. If subtropical or tropical pelagic habitats existed at the beginning or the middle of the Cainozoic, planktonic foraminifera give no clue to their whereabouts. Isotopic evidence indicates that the Oligocene was a cool period, possibly with glaciation at high latitudes⁵. More than that, however, the ungrouped faunas suggest rather structureless oceans that were not only cool, but lacking in the kind of climatic differentiation that exists now.

The Neogene radiation started at the beginning of the Miocene and by Middle Miocene there was a strong development of warm water forms. These forms, moreover, were widely distributed and, as in the Cretaceous and early Palaeogene, indicate a much greater expanse of warm water than exists today. The transition from Oligocene to Miocene cannot be dealt with now because of complex boundary questions. It is possible, however, to gain some idea of how modern plankton biogeography developed in the North Atlantic once warm water forms became re-established, by making faunal comparisons among several key areas around the North Atlantic Gyre.

The Mediterranean is a key area because it is stocked entirely from the eastern margin of the North Atlantic Gyre and its entrance is well north of the limits of the present Tropics. Since species are displaced to the south on the Ocean's eastern side, the Mediterranean is stocked with northern and subtropical forms but no tropical ones. The rare tropical forms that are found in the Mediterranean from Pliocene to Recent are not part of the indigenous fauna. On the western side of the ocean, displacement is to the north and tropical species are found as far north as 42°N, in the vicinity of the Gulf Stream⁶. Tropical forms are, however, abundantly represented in the Miocene Mediterranean, in particular, *Globorotalia menardii*, a modern tropical species with a geological record dating back to the Miocene. From its evolution in the Middle Miocene until the end of the Miocene, this species is common and continuously distributed throughout the Mediterranean and there can be no doubt that it was part of the indigenous fauna. It would seem therefore, that the Tropics extended much further north during the Miocene, at least to the vicinity of the entrance to the Mediterranean (36°N).

An alternative theory is a Miocene Tethyan seaway, with penetration of tropical forms from the Indo-Pacific. There is, indeed, much about Miocene planktonic faunas to suggest Tethyan relationships, but evidence from marine invertebrates seems to demonstrate convincingly that the eastern gateway to the Mediterranean was closed by about the middle of the Cainozoic^{7,8}.

Unfortunately, planktonic faunas from the western side of the North Atlantic are poorly known except for the tropical region where, of course, *G. menardii* is abundantly represented. The Deep Sea Drilling Project (DSDP) has, however, cored two

high latitude sites in the North Atlantic that have very important biogeographic implications. One site is on the western side of the ocean near Labrador (50°25'N), the other on the eastern side in the Hatton-Rockall Basin near the British Isles at 57°30' (ref. 9). The latter site yielded good late Miocene faunal assemblages that included *G. menardii*. Associated with *G. menardii* were the modern cold-water species *Globigerina bulloides* and *G. pachyderma* together with modern warm water, although not strictly tropical, species *Globigerinoides ruber*, *G. trilobus* and *Orbulina universa*¹⁰. This kind of mixed association is indicative of waters near the margin of the Gyre. The western drilling site near Labrador yielded a poor Miocene section, but faunal assemblages yielded warm-water forms¹¹. Also, early Miocene assemblages with close Caribbean affinities have been encountered near Nova Scotia¹².

These data, although sparsely distributed, indicate an expanded body of warm gyral water for the Miocene, the gyral limits extending perhaps 10° further north than they do now. Tropical limits probably also extended perhaps 10° further north than at present. This expanded warm gyral water persisted until the end of the Miocene, so it is unlikely that modern glaciation in the Northern Hemisphere began before the Pliocene.

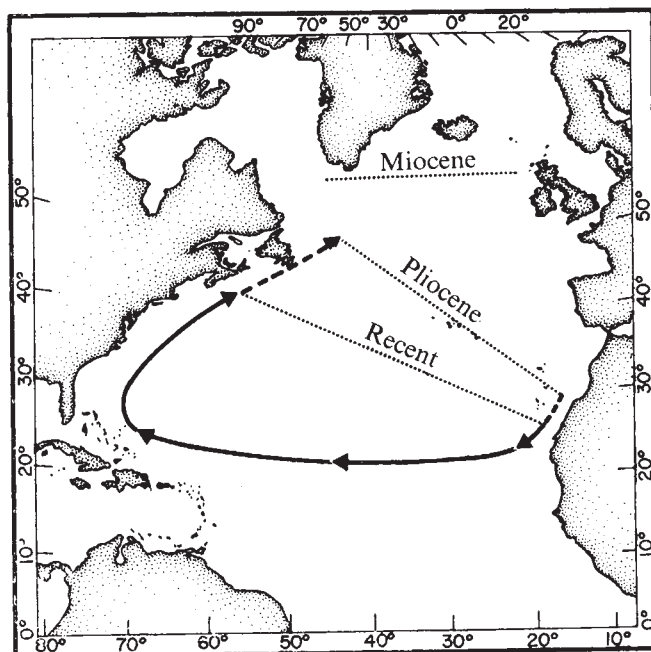


Fig. 1 Clockwise displacement of tropical species in Pliocene and Recent. During the Miocene the northern limits of tropical forms extended much further north on the eastern side of the North Atlantic and E-W distribution may have been symmetrical.

At the end of the Miocene, the Mediterranean was completely closed and there was a 'salinity crisis'. Evaporation was widespread and thick deposits of salt accumulated over the abyssal plain of the entire basin¹³. Although this evaporation must have had a disruptive effect on planktonic faunas, it was of geologically short duration, because the faunal succession across the Mio-Pliocene boundary in the Mediterranean is almost complete. A major biogeographic change is, however, associated with this boundary because the Mediterranean ceased to be part of the tropical regime at the beginning of the Pliocene¹⁴. A lack of tropical forms is evident with the first Pliocene transgression following the 'salinity crisis', but although the possible connection with the salinity crisis is not clear, the coincidence of events seems remarkable.

The disappearance of tropical forms in the Mediterranean was not an isolated regional phenomenon; it was related to a general retreat of tropical forms on the eastern side of the North Atlantic. Tropical forms are also lacking in the early Pliocene sections of the cores drilled in the Hatton-Rockall Basin and the Bay of Biscay^{9,10}. At both those sites early Pliocene

assemblages are composed of a mixture of northern and sub-tropical forms. Tropical forms do occur in abundance in the core drilled south of the Mediterranean, near the Cape Verde Islands¹⁵. On the western side of the North Atlantic, however, tropical forms still extended much further north during the early Pliocene than they do today. In the core drilled near Labrador, Poore and Berggren¹⁶ recorded *Globorotalia menardii* and other tropical species from sediments no older than early Pliocene. Early Pliocene assemblages consist of a mixture of northern, subtropical and tropical species—a Gulf Stream type of association¹⁰.

These data are sparsely distributed but they clearly fit into a pattern that shows a modern type of dispersal of tropical species, with a strong clockwise northwards displacement on the western side of the ocean (Fig. 1)⁶. There are some intriguing implications, as follows: During the early Pliocene the tropical limit on the eastern side of the North Atlantic retracted to a position south of the Mediterranean entrance, probably close to where it is now. There was no 'cold wave' however, as warm gyral water persisted on the eastern side of the North Atlantic as far north as the Hatton-Rockall Basin (57°N). Early Pliocene assemblages there contain a good representation of subtropical species. Also, the western margin of the Gyre extended perhaps 8° further north than it does today, as indicated by the assemblages recorded in the Labrador core¹⁰.

The early Pliocene therefore, displays an intermediacy between Miocene and modern conditions and most likely represents a critical phase in the evolution of modern ocean climate and circulation. At this time, it seems, the expanded Miocene body of warm gyral water began to contract to modern proportions. What occurred in the early Pliocene was clearly the beginning of a long term condition. Although there have been numerous subsequent climatic fluctuations, there has never been a large enough expansion of warm gyral water to allow the establishment of tropical planktonic foraminifera in the Mediterranean or the higher latitudes of the North Atlantic.

I have omitted any reference to tectonic theory because it is not clear how plate movements would help resolve the questions at hand. Planktonic foraminiferal development displays a curious iterative pattern difficult to account for by unidirectional shifting of plates. For example, it could be argued that the broad expanse of warm water forms seen in the late Cretaceous and Palaeogene was due to an open Tethyan seaway which promoted dispersal from the equatorial region. Yet, a comparable expanse of warm water forms can be seen in the Miocene, when the Tethyan seaway was closed.

It seems that there have been long term cycles in the development of ocean climate and circulation, with alternating expansions and contractions of warm surface water. Expansions are visible in the late Cretaceous, Palaeogene and Miocene and contractions are apparent in the early and middle Cainozoic, which may have involved a complete eradication of warm gyral water. Another contraction seems to have begun in the early Pliocene but in this case there have been no signs of disintegration of warm gyral water, as both tropical and subtropical species have survived several glacial epochs.

RICHARD CIFELLI

National Museum of Natural History,
Washington, DC 20560

Received September 28; accepted October 22, 1976.

- ¹ Cifelli, R., *Syst. Zool.*, **18**, 154 (1969).
- ² Douglas, R. G., *J. Foram. Res.*, **2**, 14 (1972).
- ³ Szczecura, J., and Pozaryska, K., *Rev. esp. Micropaleon.*, **8**, 23 (1976).
- ⁴ Bandy, O. L., *Micropaleontology*, **10**, 1 (1964).
- ⁵ Shackleton, N. J., and Kennen, J. P., *Init. Rep. Deep Sea Drilling Project*, **29**, 743 (1975).
- ⁶ Cifelli, R., and Benier, C., *J. Foram. Res.* (in the press).
- ⁷ Davies, A. M., *Tertiary Faunas*, **2**, 1 (Murray London, 1935).
- ⁸ Ekman, S., *Zoogeography of the Sea*, 1 (Sidgwick and Jackson, London, 1953).
- ⁹ Berggren, W. A., *Init. Rep. Deep Sea Drilling Project*, **12**, 965 (1972).
- ¹⁰ Poore, R. A., and Berggren, W. A., *J. Foram. Res.*, **4**, 91 (1974).
- ¹¹ Berggren, W. A. (1975).
- ¹² Bartlett, G. A., *Maritime Seds.*, **4**, 22 (1968).
- ¹³ Hsui, K. J., Cita, M. B., and Ryan, W. B. F., *Init. Rep. Deep Sea Drilling Project*, **13**, 1203 (1973).
- ¹⁴ Mazzola, G., *Proc. II Plankton Conf.*, **2**, 787 (Edizioni Tecnoscienza, Rome, 1971).
- ¹⁵ Parker, F. L., *Rev. esp. Micropaleon.*, **5**, 253 (1973).

Adaptation to an 8-h shift in living routine by members of a socially isolated community

THE proportion of the working population employed on some form of shift system has increased substantially over the past two or three decades. Expert opinion as to the optimal form of shift system is divided, however, between those who favour 'permanent' systems and those who favour 'rapidly rotating' ones. We have studied the adaptation of half the members of a socially isolated community to a permanent 8-h shift in their routine. Even in this seemingly ideal situation we failed to find complete adaptation of the ~24-h circadian rhythm in either body temperature or performance efficiency over the 10-d study period. This finding casts some doubt on the theory that permanent shift systems are optimal, and that the light-dark cycle is not important in entraining human circadian rhythms.

Circadian rhythms are now known to exist in most physiological parameters¹, and have also been found to affect the efficiency with which man performs various tasks². Performance speed on simple tasks, that place little reliance on short term memory, shows a rise throughout the day (with the exception of a more or less pronounced 'post-lunch decrement'³) and reaches a maximum at about 2000 (ref. 4). This rise parallels that found in body temperature. In contrast, the performance of more complex tasks, which rely heavily on short term memory, reaches a maximum rather earlier in the day⁵, and, with an exceptionally large short term memory load, has been shown to be negatively correlated with body temperature⁶.

In normal circumstances these rhythms are relatively constant from day to day, and are thought to be 'entrained' by a number of environmental *Zeitgeber* (cues) of which the most important seem to be the 24-h light-dark cycle and the 'social' environment⁷. There are, however, two fairly common situations in which man's circadian rhythms are disrupted, namely rapid transition from one time zone to another (the 'jet-lag' effect) and shift work. In the former case, the *Zeitgeber* in the new time zone seem to encourage the adaptation of the traveller's circadian rhythms to the new time zone, and complete adaptation of both temperature and performance rhythms typically occurs within about a

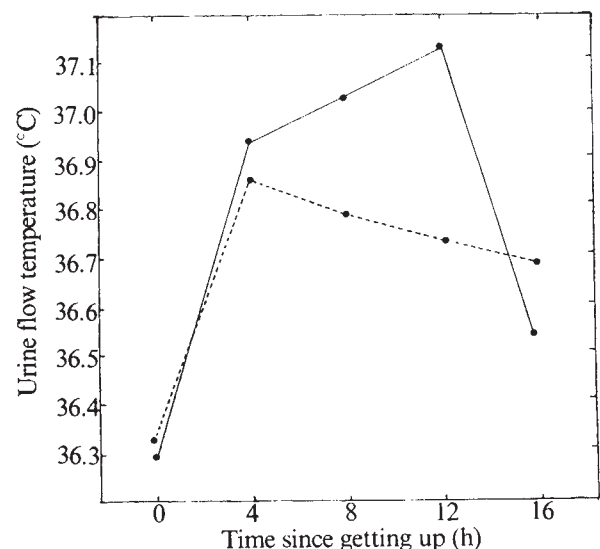


Fig. 1 The 'time since getting up' effect in urine flow temperature for the two pre-shift days (—) and for the 9th and 10th day of the shift period (---). 0, 4, 8, 12 and 16 h since getting up correspond to 0800, 1200, 1600, 2000 and 2400 for the pre-shift days, and to 1600, 2000, 2400, 0400 and 0800 for the shift period days.

week⁷. In the case of shift work, however, and in particular night shift, most *Zeitgeber* remain constant and thus discourage adaptation to the shift worked. Recent work⁸ has indicated that even after 12 successive night shifts (a rather greater number than are typically worked between rest days, during which shift workers normally revert to a 'day-time' routine) neither temperature nor performance is completely adapted to night work. Furthermore, readaptation to the normal routine during rest days seems to be very rapid and to occur within 2 or 3 d.

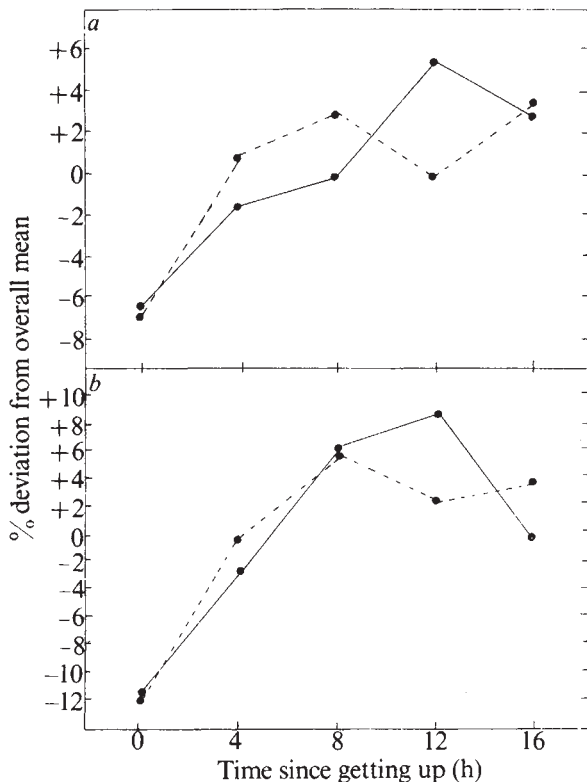


Fig. 2 The 'time since getting up' effect in *a*, manual dexterity and *b*, visual search for the two pre-shift days (—) and for the 9th and 10th day of the shift period (---). The points represent percentage deviation from the overall mean for the day in order to allow comparison between tasks.

'Permanent' night-shift systems, and slowly rotating shift systems that involve a number of successive night duties, thus result in the alternation of slow partial adaptation and rapid readaptation (during rest days) of man's circadian rhythms. In view of this, rapidly rotating shift systems (for example, two mornings, two evenings, two nights and two off) are recommended by some experts. These result in little disruption of the circadian rhythm, but result in the night work being carried out when performance, on at least simple tasks, is at low ebb. Others favour the idea of a permanent 'night subsociety' in which night workers would remain on an inverted routine even on their days off, and should thus be able to adapt completely to the night shift. This view assumes the social *Zeitgeber* to be rather more important than the light-dark cycle, and there is some evidence that this is the case⁷. It would, however, involve a considerable proportion of the population shifting the whole of their normal routine by, for instance, 8 h. This is in contrast to the present habit of most night workers who go to bed immediately after their work, and take their leisure time at the beginning of their waking day.

The present study took advantage of the complete social isolation, but normal light-dark cycle (sunrise ~ 0600, sunset ~ 1530 at the time of the study) that exist during the winter months at South Georgia. Six of the 12 members of

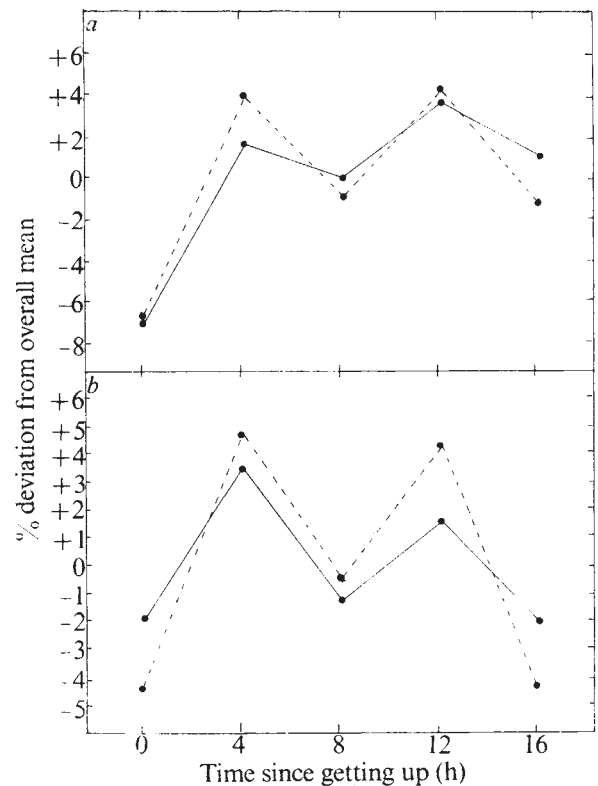


Fig. 3 The 'time since getting up' effect in *a*, arithmetic and *b*, verbal reasoning for the two pre-shift days (—) and for the 9th and 10th day of the shift period (---). The points represent percentage deviation from the overall mean for the day. In spite of considerable practice on these tasks before the study, there was evidence of a practice effect in the pre-shift results. The curves have been adjusted to take account of this using a linear approximation.

a British Antarctica Survey camp at South Georgia (54°17'S, 36°30'W) took part in the study. For ten successive days they shifted the whole of their normal daily routine by 8 h, so that instead of sleeping from 2400 to 0800 they slept from 0800 to 1600. For 2 d before, and for the last 2 d of, this 10-d period they undertook four tests of performance efficiency, and measured their urine flow temperatures⁹, at 4-h intervals throughout their waking day. Two of the performance tests used involved a negligible short term memory load: these were a 90-s visual search test involving a search through lines of upright Os for slightly tilted ones, and a manual dexterity test that involved exchanging nuts and bolts attached to two brass plates. The other two tests involved an intermediate memory load and were a 3-min test of verbal reasoning³, and a 5-min test of arithmetic³.

Since the main concern of the study was whether or not there was complete adaptation (as opposed to some adaptation), the reference point used in the analysis was the time of getting up rather than clock time. If perfect adaptation had occurred there should be no difference in the 'time since getting up' effect from the two stages of the study. If, however, the opposing light-dark cycle, and the social influences of the six members of the camp who did not shift their routine, had prevented complete adaptation then this effect from the two stages should differ.

Considerable adaptation of the temperature rhythm took place, since the two curves in Fig. 1 are not too dissimilar. Indeed, there was an overall 'time since getting up' effect (Anova $P < 0.001$), but there was also evidence that this effect differed for the two stages of the study ($P < 0.025$). Deviation from perfect adaptation at the end of the shift period took the form of a reduction in amplitude, an earlier peak, and the lack of a predictive drop before the sleep

period. As such, it is very similar to that found in earlier shift studies^{7,8}.

The two low memory load tests, manual dexterity and visual search, showed a similar, but somewhat smaller, lack of adaptation (Fig. 2). Although there was no reduction in the amplitude of the 'time since getting up' effect, performance on both tasks reached an earlier peak and failed to show a drop before sleep at the end of the shift period. Again, considerable adaptation had clearly occurred, and only in the case of visual search was there evidence that this adaptation was incomplete ($P < 0.025$).

The two intermediate memory load tasks showed even better adaptation (Fig. 3), and for neither task was there any evidence that the adaptation was incomplete ($P > 0.25$ in both cases). Performance on both tasks reached an early peak 4 h after getting up, and a second peak 8 h later. This double peak has not normally been found⁴, although it has been noted before with an intermediate memory load task⁶. It seems to represent a composite of the early peak shown by pure short term memory tasks, and the late peak shown by simple tasks. The slightly more exaggerated 'time since getting up' effect that these two intermediate load tasks showed at the end of the shift period may be due to partial sleep deprivation. As is typical for night workers¹⁰, some of the present subjects complained of their inability to sleep properly during the shift period, and there is some evidence that sleep deprivation results in more marked daytime effects³. The reason such an exaggeration was not found in the simple tasks may be because such tasks are generally less prone to sleep-deprivation effects¹¹.

The conclusions to be drawn from these results are, in general, very similar to those based on studies where the subjects have not been members of a socially isolated community⁸. Even after ten successive days of an 8-h shift, complete adaptation had not occurred in either temperature or visual search performance. Although the other performance measures showed no significant lack of adaptation, it is generally accepted that different rhythms adapt to a time shift at different rates⁷, and the adaptation of performance rhythms may well be quicker than that of the physiological ones. The lack of complete adaptation could be due to the social influences of the six members of the camp who did not shift their routine, and/or to the opposing light-dark cycle. Although it has recently been suggested that the light-dark cycle is relatively unimportant for man⁷, this is based on results using only an artificial light-dark cycle. It is quite possible that the natural cycle is a rather stronger *Zeitgeber* than these studies would suggest, especially since in the present study the contact between the shifted and unshifted members of the camp was minimal.

D.G.H. thanks his colleagues in Antarctica for their willing help and cooperation in this project.

D. G. HUGHES

Alderhay Hospital,
Liverpool L12 2AI, UK

SIMON FOLKARD*

MRC Perceptual and Cognitive Performance Unit,
Experimental Psychology Laboratory,
University of Sussex,
Brighton, Sussex BN1 9QG, UK

Received August 6; accepted October 20, 1976.

*To whom reprint requests should be sent.

¹ Conroy, R. T. W. L., and Mills, J. N., *Human Circadian Rhythms* (Churchill, London, 1970).

² Blake, M. J. F., *Psychon. Sci.*, **9**, 349-350 (1967).

³ Colquhoun, W. P., *Biological Rhythms and Human Performance* (edit. by Colquhoun, W. P.), 37-107 (Academic, London, 1971).

⁴ Hockey, G. R. J., and Colquhoun, W. P., *Aspects of Human Efficiency—Diurnal Rhythm and Loss of Sleep*, (edit. by Colquhoun, W. P.), 1-23 (English Universities Press, London, 1972).

⁵ Folkard, S., *Br. J. Psychol.*, **66**, 1-8 (1975).

⁶ Folkard, S., Knauth, P., Monk, T. H., and Rutenfranz, J., *Ergonomics*, **19**, 479-488 (1976).

⁷ Aschoff, J., Hoffmann, K., Pohl, H., and Wever, R., *Chronobiologia*, **2**, 23-78 (1975).

⁸ Colquhoun, W. P., Blake, M. J. F., and Edwards, R. S., *Ergonomics*, **11**, 437-453 (1968).

⁹ Fox, R. H., Woodward, P. M., Fry, A. J., Collins, J. C., and MacDonald, J. C., *Lancet*, **i**, 424-427 (1971).

¹⁰ Knauth, P., and Rutenfranz, J., *Experimental Studies of Shift Work* (edit. by Colquhoun, W. P., et al.), 57-65 (Westdeutscher Verlag, Opladen, 1975).

¹¹ Wilkinson, R. T., *Aspects of Human Efficiency—Diurnal Rhythm and Loss of Sleep* (edit. by Colquhoun, W. P.), 25-30 (English Universities Press, London, 1972).

Short term increases in mortality during heatwaves

COMPARISON of the weekly death registrations in Greater London¹ with temperatures at the London Weather Centre during the summer of 1975 shows a temporary increase in mortality that coincides with the heatwave of late July to mid-August (Fig. 1). There was also an increase in death registrations in the first week of June, when there was a change from cold weather with a little snow to very warm weather (but these figures may have been slightly inflated by increased delays in registration following a public holiday in the previous week). Increases in mortality associated with heatwaves have been widely reported in North America, although there is some evidence that the pattern there may have been modified by air conditioning²⁻³. Little attention has been drawn to such episodes in England and Wales, although an association between deaths from cerebral infarction and temperature in summer has been reported⁴.

The hot weather during the summer of 1975 was exceptional, yet similar conditions were again recorded in 1976 (Fig. 2). During the heatwave in late June and early July, temperatures were marginally higher than in 1975. Again there was an increase in weekly death registrations in Greater London, predominantly among the over-65 age group. This heatwave affected much of the country; attention has been drawn to a parallel rise in deaths in the remainder of south-east England⁵, and mortality increased for England and Wales as a whole. There was also a small increase in deaths in early May (Fig. 2), when temperatures were high for the time of year. The reduced number of registrations in the week ending September 2 was probably attributable to delays following the public holiday in that week: any similar tendency in 1975 may have been obscured by the further rise in temperature that occurred then.

There are clearly many difficulties in the interpretation of short term changes in mortality based on weekly registration records. This is particularly true in relation to the effects of hot periods, which in England are usually short, although some other major episodes (in 1974 for example), have been associated with marked increases in weekly totals. For a period of hot weather in 1968, however, death totals for Greater London, tabulated by date of occurrence, have been provided by the Office of Population Censuses and Surveys, as part of a general arrangement for a larger study of the effects of environmental factors on mortality (A.M. unpublished). Figure 3 shows the death totals together with daily temperatures measured at London Airport (Heathrow) for this period. Weather data from Heathrow were selected for the main study because of their availability on magnetic tape.

The hot period at the beginning of July was comparatively short lived, and it was associated with warm air currents from the Sahara, which brought an unusually warm night (minimum 20 °C), during which a layer of red dust fell over much of southern and eastern England. This was followed on July 1 by an exceptionally warm day (maximum 32 °C), and another warm night. Similar temperatures were recorded at the London Weather Centre and elsewhere within and beyond Greater London. Mortality increased sharply on July 1 and remained high on the following day. Increases were seen in cardiovascular

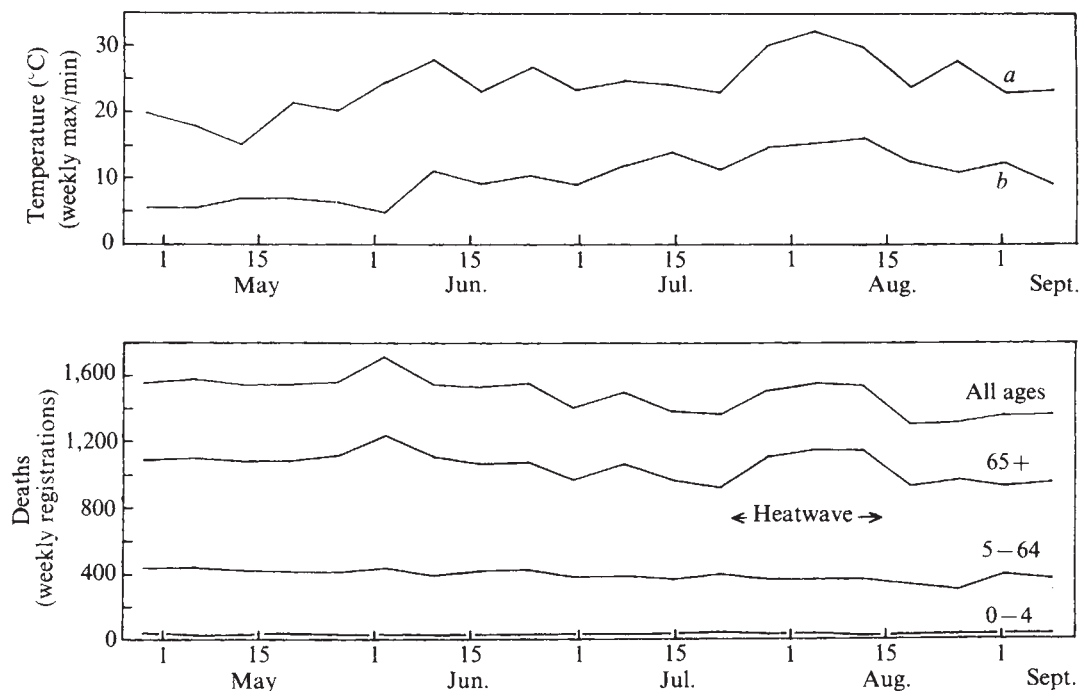
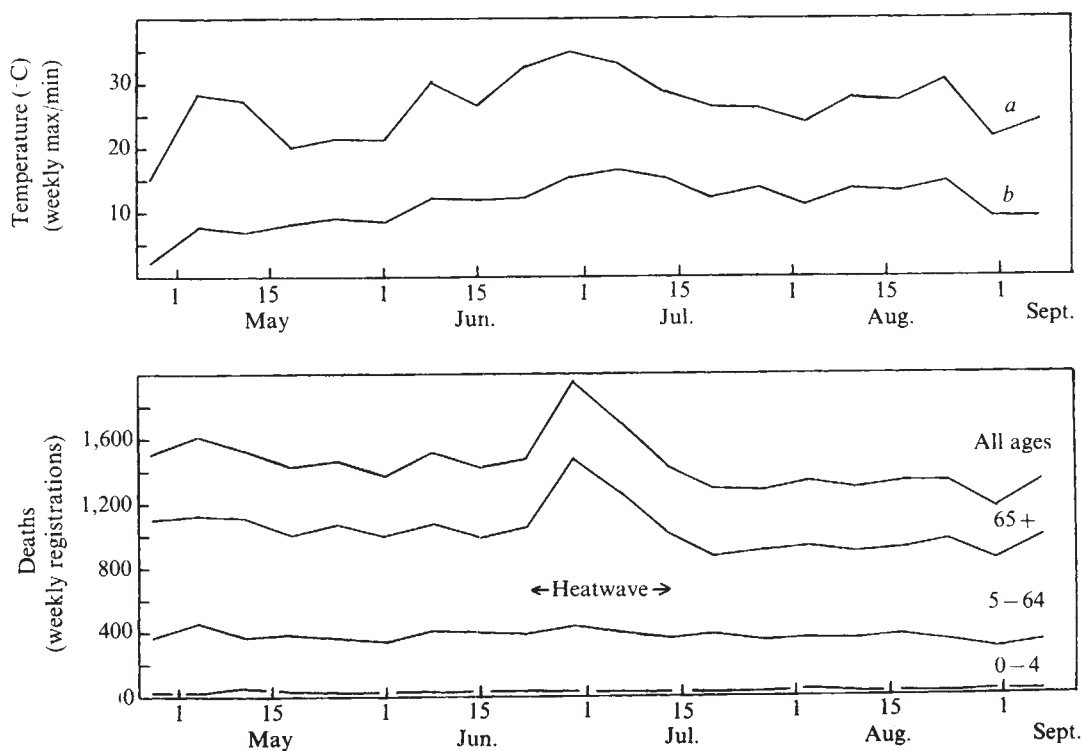


Fig. 1 Weekly totals of deaths registered in Greater London, by age, and weekly maximum (a) and minimum (b) temperatures (London Weather Centre), for summer 1975.

and respiratory causes of death, and in the total of the remaining causes. It is unlikely that the dust associated with this heatwave had any direct effect on mortality. No complete study of the particle size distribution of the dust in suspension was reported, but the mass median diameter

of deposited particles was found to be $9\mu\text{m}$ in a study in Hull⁸. This is consistent with unpublished results obtained in London, and these findings indicate that much of the material was too coarse to be inhaled deeply into the lungs.

Fig. 2 Weekly totals of deaths registered in Greater London, by age, and weekly maximum (a) and minimum (b) temperatures (London Weather Centre), for summer 1976.



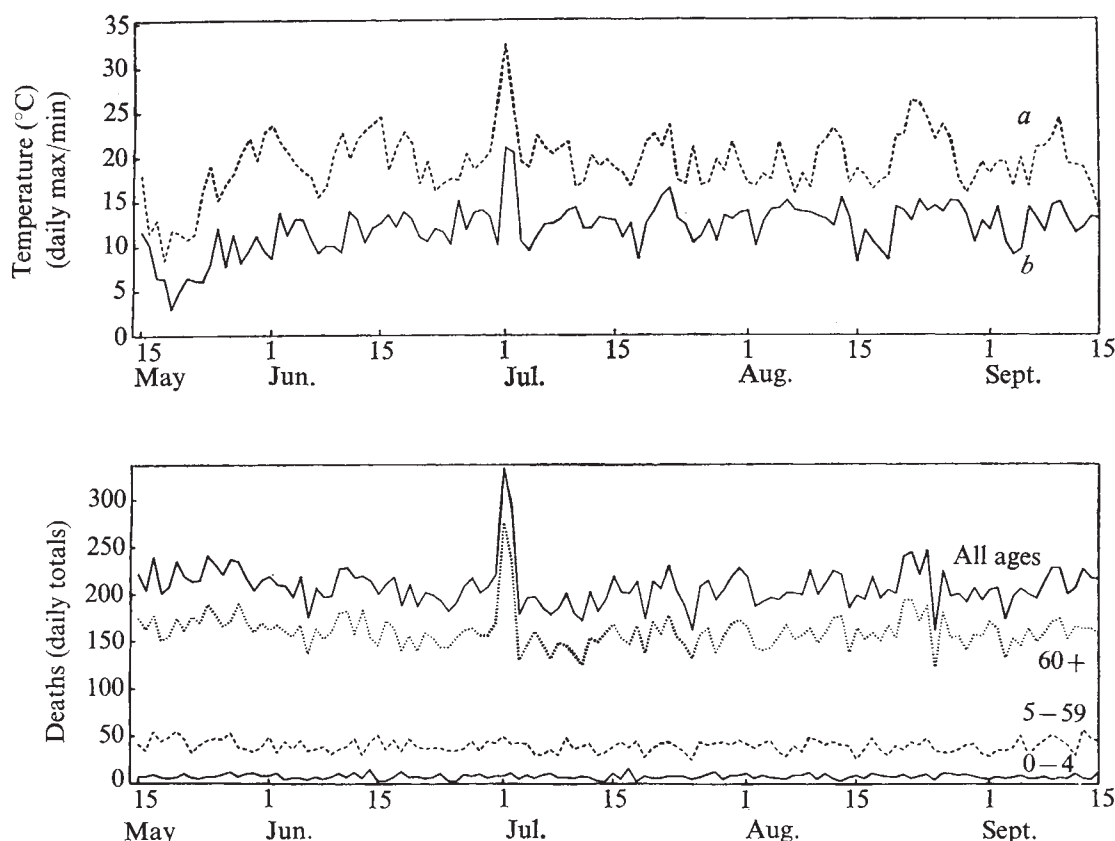


Fig. 3 Daily totals of deaths occurring in Greater London, by age, and daily maximum (a) and minimum (b) temperatures (London Airport, Heathrow) for summer 1968.

An analysis by age at death is included in Fig. 3. Increases in mortality were seen both in the 60–74 and 75+ age groups, but here the two have been combined for clarity of presentation. There was no sign of any increase in mortality in the 0–4 age group (unfortunately it was not possible to subdivide this group retrospectively), in contrast to findings in the United States of increases in infant mortality during heatwaves. The number of deaths per day in this age group in Greater London is, however, very small; it would probably have been necessary to obtain data from a larger population to detect any significant increase in infant mortality.

In other years since 1968, associations have been seen between rises in temperature and increases in daily mortality in the summer months on occasions when the meteorological changes were less dramatic than those described here, and the increases in mortality were smaller; indeed, they were not evident in the weekly death registration totals, and only became evident when the daily totals were examined. This work will be reported in detail elsewhere.

ALISON MACFARLANE

Department of Medical Statistics and Epidemiology,
London School of Hygiene and Tropical Medicine,
Keppel Street, London WC1E 7HT, UK

R. E. WALLER

MRC Environmental Hazards Unit,
St Bartholomew's Hospital Medical College,
London EC1M 6BQ, UK

³ Ellis, F. P., *Environ. Res.*, 5, 1–58 (1972).

⁴ Marmor, M., *Archs. environ. Hlth*, 30, 130–136 (1975).

⁵ Oechsli, F. W., and Buechley, R. W., *Environ. Res.*, 3, 277–284 (1970).

⁶ Bull, G. M., and Morton, J., *Age and Ageing*, 4, 19–31 (1975).

⁷ Lyster, W. R., *Lancet*, ii, 469 (1976).

⁸ Pitty, A. F., *Nature*, 220, 364–365 (1968).

Mycorrhizal fungi stimulate clover growth in New Zealand hill country soils

VESICULAR-ARBUSCULAR mycorrhizal fungi infect most higher plants and usually increase plant growth and phosphorus uptake, especially in infertile soils¹. Mosse^{2–4} has shown that the indigenous mycorrhizal fungi in some soils are very infective but much less efficient in stimulating phosphorus uptake and plant growth than selected mycorrhizal fungi maintained in glasshouse pot culture. New Zealand soils have a short history of pastoral agriculture (<150 yr) and still range in fertility from very phosphorus-deficient podocarp-broadleaf forest soils to well developed and highly fertile pasture soils. Most hill country pastures consist of introduced grasses and legumes which are invariably mycorrhizal^{5,6}. These pastures have been developed within the past 100 yr from infertile forest soils in which nearly all plants were mycorrhizal⁷ and many were completely dependent on mycorrhizal infection for phosphate uptake and plant growth^{8,9}. Many forests are still being cleared and brought into pasture by oversowing with white clover and spreading heavy dressings of superphosphate. It is important to know whether the indigenous mycorrhizal fungi adapted to these infertile forest soils are as effective at stimulating clover growth in highly fertilised pastures as an efficient mycorrhizal fungus such as *E₃* (ref. 10), a *Glomus* species from Mosse's Rothamsted collection.

Received July 5; accepted October 14, 1976.

¹ OPCS Monitors (Office of Population Censuses and Surveys, England and Wales, London, 1975).

² Gover, M., *Pub. Hlth Rep. (US)*, 53, 1122–1143 (1938).

Table 1 Hill country soils* in which E_3 was more efficient than the indigenous mycorrhizal fungi

Soils	Soil P† (p.p.m.)	No fungi (controls)	Shoot DM (mg) of white clover plants infected with different mycorrhizal fungi		LSR ($P = 0.05$)
			Indigenous fungi	E_3	
Taihape silt§	8	30	65	242	1.73
Marua clay†	8	10	75	119	2.74
Marua clay§	12	6	115	118	1.63
Waingaro steepeland‡	12	11	249	348	1.94
Te Kuiti silt§	12	10	134	315	1.74
Te Pari silt†	12	8	308	413	1.62
Te Pari silt§	24	110	511	944	1.56
Te Pari silt§	32	61	353	734	1.51
Autea clay†	12	17	27	235	1.65
Autea clay§	24	15	117	257	1.92
Autea clay§	24	22	402	557	2.71
New Plymouth brown loam§	16	20	98	235	2.13
Patua loam†	16	24	305	547	2.06
Patua loam¶	28	18	665	673	2.86
Burrell sandy loam‡	16	41	36	702	1.61
Mahoenui silt‡	16	77	199	367	1.54
Mahoenui silt§	88	607	657	854	1.48
Gisborne sandy loam‡	16	25	122	297	3.29
Gisborne sandy loam¶	60	312	476	737	1.70
Mamaku sand¶	20	6	61	97	1.52
Waitakere hill§	24	32	468	634	1.62
Marua hill†	32	329	500	666	1.93
Waikare clay†	48	193	389	393	1.49
Dunmore silt§	120	569	604	682	1.57

*All soils sterilised.

†Measured by modified Truog test¹².

‡Soil from broadleaf forest.

¶Soil from recent or rough pasture.

§Soil from well developed pasture.

Data analysed by analysis of variance after log transformation. When the ratio of any two shoot DM means $\geq$ LSR (least significant ratio), the means are significantly different at $P = 0.05$.

Accordingly, samples of 37 hill country soils were collected in the North Island of New Zealand, in several instances from adjacent forest, recent pasture and well-developed pasture sites on the same soil type. Soils were sieved and most of the sample sterilised with methyl bromide to kill the indigenous mycorrhizal fungi. Most soils had a pH between 5.2 and 5.8. White clover was grown from seed in the unsterilised portion of each soil and at intervals seedlings were harvested and their roots cleared and stained to assess the speed of infection of the indigenous mycorrhizal fungi.

In three infertile forest soils (Waingaro steepeland, Waitakere hill, Patua loam) clover roots were only 12, 2 and 1% mycorrhizal 15 d after seed germination, but infection levels gradually rose to 40% after 43 d. In two other forest soils (Autea clay, Burrell sandy loam) the indigenous fungi also infected very slowly with only 6–11% of the roots mycorrhizal even after 43 d. In all the remaining soils, including the Waitakere, Patua, Autea and Burrell soils from under well developed pasture, the indigenous mycorrhizal fungi in the pasture were very infective, with 30–90% of clover roots mycorrhizal within 15 d of seed germination. It is apparent therefore, that during the change from forest to pasture, soils not only become more fertile (Table 1) but their mycorrhizal fungi infect clover more rapidly.

In each of the 37 soils, clover was grown from seed for one month in the unsterilised portion, and seedlings became infected with the indigenous mycorrhizal fungi. Seedlings were then transplanted two per pot into 10-cm pots of the same soil which had been sterilised.

For comparison, seedlings which had been raised in E_3 -infested sterilised soil and control seedlings in sterilised soil with no added fungi were also transplanted into pots of each sterilised soil. Twice filtered washings from all unsterilised soils were added to the pots to ensure that all plants received the same microflora apart from mycorrhizal fungi.

All plants were inoculated twice with *Rhizobium trifolii* strain TA₁ and received weekly applications of nutrient solution containing all essential elements except N and P. This ensured that P alone was limiting growth. There were four replicate pots of each of the 111 treatments (37 soils $\times$ 3 mycorrhizal regimes) randomly arranged and the experiment ran for eight weeks in a shaded glasshouse.

Tables 1 and 2 show that most of the indigenous mycorrhizal fungi were quite efficient and greatly increased growth over controls, especially in the infertile forest soils in which responses were up to 38-fold (Te Pari silt loam, 12 p.p.m. P, Table 1). In all but four soils, 30% or more of the root system was mycorrhizal.

In Burrell sandy loam (forest, 16 p.p.m. P) however, the indigenous fungi had only infected 8% of clover roots and mycorrhizal plants were smaller than controls. In Autea clay (forest, 12 p.p.m. P), the indigenous fungi had managed to infect 39% of clover roots, but were very inefficient and hardly increased shoot DM over controls. In both these soils, inoculation with E_3 led to massive increases in shoot dry matter (DM) (Table 1). In the other three forest soils in which the indigenous mycorrhizal fungi had been initially slow to infect, root infections were high and growth responses to indigenous mycorrhizal fungi were large.

Although the response to mycorrhizal infection decreased as soil fertility rose (Tables 1 and 2) the indigenous mycorrhizal fungi were still beneficial in most of the fertile soils, with a growth response of 39% occurring in Burrell sandy loam from pasture (120 p.p.m. P, Table 2). In five soils, the growth responses to the indigenous mycorrhizal fungi were not significant at the 5% level although they followed the general trend.

In 24 soils, including most forest soils, E_3 was more efficient than the indigenous mycorrhizal fungi at increasing plant growth (Table 1). E_3 infection caused 1,940% more plant growth than the indigenous mycorrhizal fungi in Burrell sandy loam from under forest (16 p.p.m. P, Table

Table 2 Hill country soils* in which the indigenous mycorrhizal fungi were more efficient than E₃

Soils	Soil P (p.p.m.)	Shoot DM (mg) of white clover plants infected with different mycorrhizal fungi			LSR (P = 0.05)
		No fungi (controls)	Indigenous fungi	E ₃	
Te Kuiti silt§	12	10	355	156	1.59
Whangamomona hill§	12	12	269	151	1.75
Waitakere hill‡	16	62	432	408	1.71
Marua hill§	20	90	459	369	1.99
Aria silt§	24	52	866	707	1.97
Patua loam§	44	37	783	352	2.19
Oruanui hill§	44	137	852	837	1.88
Marua hill¶	56	33	528	498	2.79
Mahoenui silt§	48	447	1,013	667	1.42
Oropi sandy loam§	64	759	1,268	997	1.42
Waikare clay§	108	597	727	631	1.28
Gisborne sandy loam§	108	1,060	1,230	1,169	1.31
Burrell sandy loam§	120	988	1,373	1,139	1.39

*All soils sterilised.

‡, ¶, § as in Table 1.

1), and in excess of 100% more growth than the indigenous fungi in seven other soils, and 30% more growth in a total of 20 soils (Table 1).

The efficiency of a mycorrhizal fungus at increasing plant growth was not related to the percentage of host roots it infected. For example, in Taihape silt loam the E₃-infected plants had 55% of roots mycorrhizal and a mean shoot DM of 242 mg, while plants infected with the indigenous mycorrhizal fungi had 73% of mycorrhizal roots but only 65 mg shoot DM. For most soil types, the increased efficiency of E₃ over the indigenous mycorrhizal fungi was greatest in the forest soil, but in Te Pari silt loam, the reverse was true.

In 11 soils the indigenous fungi were more efficient than E₃ (Table 2), but in only four soils (Te Kuiti, Whangamomona, Patua and Mahoenui) was the increased growth greater than 30%.

In conclusion, it is apparent that clover is highly dependent on infection by mycorrhizal fungi for growth in many hill country soils. The extra benefit from E₃ over the indigenous mycorrhizal fungi achieved in such a wide range of soils is very promising, and field inoculation may be feasible if E₃ can be established in natural soils in the face of competition from the indigenous but less efficient fungi already present. Mosse⁴ has already demonstrated this in some British soils.

I am grateful to Mr Jack Park and Mrs Jeanette Daniel for technical assistance, and Mr M. B. O'Connor for selecting suitable soils.

CONWAY POWELL

Ruakura Agricultural Research Centre,
Hamilton, New Zealand

Received August 13; accepted September 28, 1976.

- ¹ Mosse, B., *A. Rev. Phytopath.*, **11**, 171–196 (1973).
- ² Mosse, B., Hayman, D. S., and Ide, G. J., *Nature*, **224**, 1031–1032 (1969).
- ³ Mosse, B., and Hayman, D. S., *New Phytol.*, **70**, 29–34 (1971).
- ⁴ Mosse, B., in *Endomycorrhizas* (Academic London, 1975).
- ⁵ Crush, J. R., *N.Z. J. Bot.*, **11**, 645–660 (1973).
- ⁶ Crush, J. R., *N.Z. J. agric. Res.*, **18**, 361–364 (1975).
- ⁷ McNabb, R. F. R., thesis, Otago Univ., New Zealand (1958).
- ⁸ Baylis, G. T. S., *New Phytol.*, **66**, 231–243 (1967).
- ⁹ Baylis, G. T. S., in *Endomycorrhizas* (Academic, London, 1975).
- ¹⁰ Mosse, B., Powell, C. L., and Hayman, D. S., *New Phytol.*, **76**, 331–342 (1976).
- ¹¹ Phillips, J. M., and Hayman, D. S., *Trans. Br. mycol. Soc.*, **55**, 158–161 (1970).
- ¹² Moutier, N. S., Grigg, J. L., and Oomen, G. A. C., *N.Z. J. agric. Res.*, **9**, 328–338 (1966).

Protozoa as sources of antigen in 'humidifier fever'

IN the field of hypersensitivity reactions to inhaled materials, sources of antigenic production are as varied as avian protein, pituitary stuff, wheat weevil, *Aspergilli*, and thermophilic actinomycetes¹. We have found a completely new group of organisms

which give rise to antigens associated with 'humidifier fever'² one of the diseases in the extrinsic allergic alveolitis group¹. The organisms are amoebae that develop in the recirculating water used in the humidification systems.

The ubiquitous nature of these Protozoa suggests that sensitisation to them or their products may not be confined to the groups of individuals already studied, and that industry should be made aware of the possibility of the development of these organisms in stagnant or recirculating water, particularly when processes release material upon which microorganisms can develop into the environment.

Our attention was first drawn to the problem when about 20 of 50 office staff were reported as having pyrexial episodes, often with myalgia, some hours after leaving the factory, and usually on a Monday. The office adjoins the factory which uses a humidifier for plant heating as well as for one of its industrial processes. Moreover, above the office is a suspended ceiling on which dust from the factory has accumulated.

Such episodes have been recorded previously as 'humidifier fever'² or as hypersensitivity pneumonitis attributable to air conditioner contamination,³ although the clinical manifestations are not necessarily identical with those we have found.

It is considered that contamination of the humidifiers by organisms, and the release of these or their products into the environment is responsible for sensitisation and subsequent disease⁴. The source of antigens has been described as a member of the *Micropolyspora* genus³ (now identified as *Thermoactinomyces vulgaris*—J. Salvaggio, personal communication), *T. vulgaris*⁴ or thermotolerant bacilli⁵. Lines of precipitation have been recorded against *T. vulgaris* with sera from affected individuals⁴, and inhalation challenge tests using *T. vulgaris* have produced a pyrexial episode^{3,4}. However, some *T. vulgaris* strains can produce materials capable of reacting nonspecifically with human sera⁶, as can material from *Staphylococcus aureus*⁷ and the so-called byssinosis 'antigen'⁸. Apart from this possibly 'false positive' reaction, serological testing with extracts of other organisms has been without success^{9,10}, although lyophilised water from the humidifier may contain materials capable of reacting with sera of affected individuals¹⁰.

Our studies involved isolating organisms from the atmosphere of the office, the dust on the false ceiling, and from the humidifier. Many fungi and bacteria were isolated, identified, and extracts were tested against sera from affected individuals. Apart from one or two weakly reacting extracts, results were negative on gel diffusion, although extracts of the 'ceiling dust' and material from the humidifier baffle plates reacted strongly with the sera of 16 of 18 affected individuals and 2 of 18 unaffected individuals working in the office ($P < 0.001$).

When material from the baffle plates was viewed microscopically it became apparent that Protozoa were present in abundance. Isolation provided two main groups of

Protozoa—ciliates and amoebae. Purified ciliates failed to produce antigenic material that reacted with sera from affected cases, whereas amoebae produced up to five precipitin lines. A named amoeba, *Naegleria gruberi* was obtained (Culture Centre of Algae and Protozoa, Cambridge), and grown in monoxenic culture with *Klebsiella aerogenes*. The resulting extract was antigenic, and the gel diffusion reaction indicated antigenic identity with material in the ceiling dust (Fig. 1). Amoebae isolated from the ceiling dust produced a similar result; *K. aerogenes* extract was weakly positive.

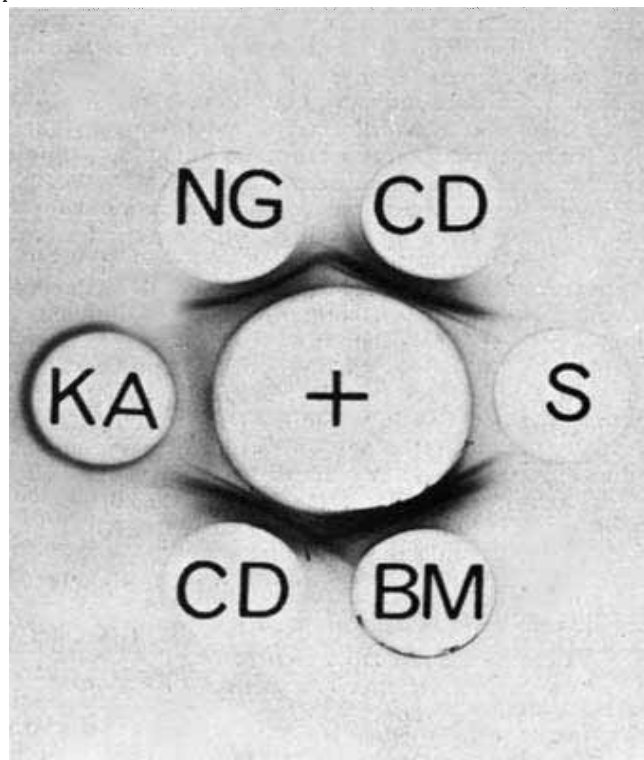


Fig. 1 Gel diffusion reaction: +, pooled sera from affected cases; S, saline; CD, ceiling dust extract; BM, Baffle plate 'muck' extract; KA, *Klebsiella aerogenes* extract; NG, *Naegleria gruberi* extract; CD and BM were aqueous extracts of ceiling dust and baffle plate muck concentrated and dialysed.

N. gruberi was grown for 7 d at room temperature on non-nutrient agar streaked with live *K. aerogenes*¹¹ and the plates extracted with 1% aqueous phenol, then concentrated and dialysed. *K. aerogenes* was prepared by the same method as *N. gruberi*. Note the join of the line from *N. gruberi* and CD indicating antigenic identity.

Thus, we believe we have been able to determine a source of antigens associated with 'humidifier fever' that has hitherto remained unrecognised; possibly one species of amoeba, that is, *N. gruberi*, will not provide all the antigens to which affected cases respond serologically; indeed, our current work suggests that several amoebae are present. We hope to assess the individual contribution of each of these to the overall antigenic picture.

We are indebted to Drs A. Jones and G. Westhall of the E.M.A.S. for first bringing the cases to our attention, and to Dr A. Axford for his help in clinical epidemiology. The photographic expertise of D. Llewellyn is appreciated.

MRC Pneumoconiosis Unit,
Penarth, Glamorgan

Department of Microbiology,
University College Cardiff,
Glamorgan

Asthma Research Unit,
Sully Hospital, Penarth,
Glamorgan, UK

Received August 5; accepted September 29, 1976.

¹ Pepys, J., *Monographs on Allergy*, vol. 4 (Karger, Basel, 1969).

² Pestalozzi, C., *Schweiz. med. Wschr.*, **89**, 710–713 (1959).

³ Banaszak, E. F., Thiede, W. H., and Fink, J. N., *New Engl. J. med.*, **283**, 271–276 (1970).

⁴ Fink, J. N., *et al.*, *Ann. Int. Med.*, **84**, 406–413 (1976).

⁵ Kohler, P. F., Gross, G., Salvaggio, J., and Hawkins, J., *Chest*, **69S**, 294–296 (1976).

⁶ Edwards, J. H., Baker, J. T., and Davies, B. H., *Clin. Allergy*, **4**, 379–388 (1974).

⁷ Forgren, A., and Sjoquist, J., *J. Immun.*, **97**, 822–827 (1966).

⁸ Edwards, J. H., and Jones, B. M., *J. Immun.*, **110**, 498–501 (1973).

⁹ Keller, H., Spengler, H., and Latscha, U., *Schweiz. med. Wschr.*, **102**, 865–872 (1972).

¹⁰ Pickering, C. A. C., Moore, W. K. S., Lacey, J., Holford-Strevens, V. C., and Pepys, J., *Clin. Allergy*, **6**, 109–118 (1976).

¹¹ Page, F. C., *Protistological*, **11**, 195–204 (1975).

Suppression of murine lupus erythematosus by Dactinomycin

THE disease of the NZB×NZW/F₁ hybrid mouse (B/W) is a model of systemic lupus erythematosus (SLE), perhaps the most autoimmune of human diseases. The animals develop LE cells and anti-nuclear antibodies of various specificities, and die of a fulminating chronic glomerulonephritis at an early age¹. Abnormalities of the skin² and central nervous system³, also reminiscent of clinical SLE, are present as well. Like SLE patients, many of the mice have greatly extended lifespans under treatment with corticosteroids, azathioprine or cyclophosphamide, or combinations thereof^{1,4}. There is a body of evidence⁵ which suggests that aberrant availability of autoantigens may be a factor in both SLE and B/W disease. Other groups have emphasised the availability of nuclear materials from keratinising skin cells as a source^{6–8}, but it seems to us that the nuclei from maturing erythrocytes should also be considered. All of the immunosuppressive agents mentioned above suppress erythropoiesis in mice⁹ and may also be limiting access of

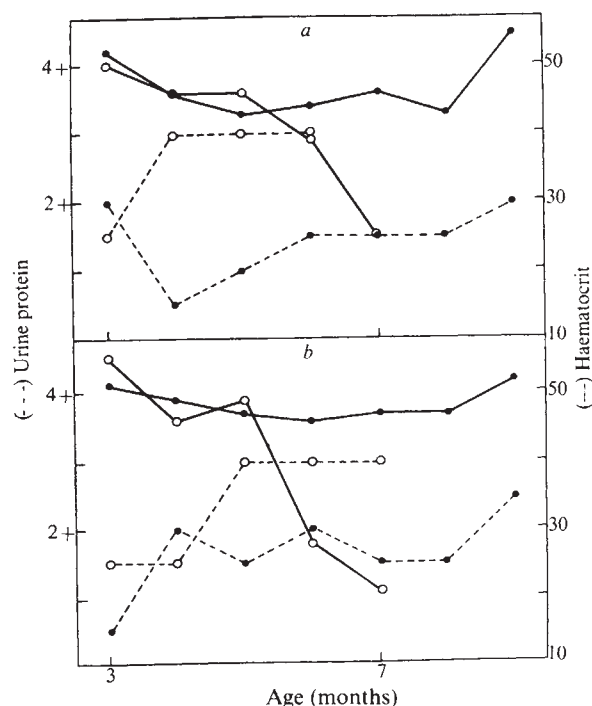


Fig. 1 Longevity of Dactinomycin-treated NZB/NZW mice to 405 d. Control deaths began at about 6 months of age, and the last control died at close to 12 months. The cut-off on the graph was arbitrary, but all treated mice were alive beyond 450 d.

antigen to immune complexes. Here we show that Dactinomycin (actinomycin D), which inhibits erythropoiesis at doses which do not suppress antibody formation (ref. 9 and unpublished observations), is nevertheless an effective suppressant of B/W mouse disease.

In the present series female B/W mice were treated with Dactinomycin, beginning with a small daily dose, 0.35 μg subcutaneously, at about 3.5 months of age, increased to 1.8 $\mu\text{g d}^{-1}$ about 6 weeks later and doubled to 3.5 $\mu\text{g d}^{-1}$ a month after that, at about 6 months of age. The adjustments reflected significant reticulocyte counts in most of the treated animals. Weight was checked twice weekly; and

haematocrit, reticulocyte count, and urine protein every 4–5 weeks. The drug was stopped when weight loss was consistent in the group; treatment was suspended for an average of 5–6 d a month. Post-mortem tissues were fixed and snap-frozen for light and fluorescence microscopy.

At the present time, all 11 Dactinomycin-treated animals are alive at 14 months of age or more, mean 455 d (range 444–460), while all 11 age-matched control animals have died at a mean age of 256 d (range 189–358 d). Figure 1 illustrates the longevity data to 405 d. The difference is significant by the sign test ($P < 0.001$)¹⁰ and by the Mann-Whitney test ($P < 0.01$)¹¹. The treated animals have generally had low (or no) reticulocyte counts, but have maintained haematocrits at 40 or above. Urine protein at 14 months of age was 2+ and 3+ in single animals, but less in the other nine.

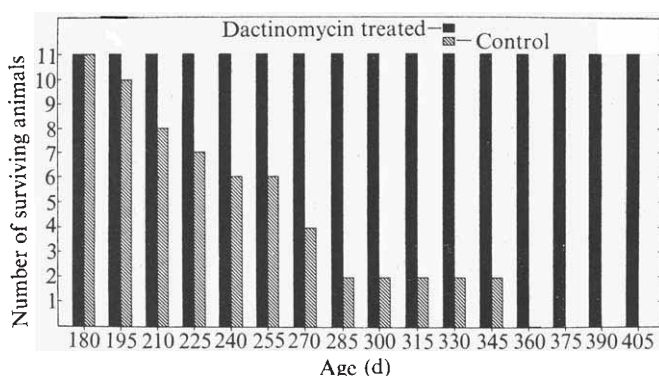


Fig. 2 Urine protein (-----) and haematocrit (—) in matched Dactinomycin-treated (●) and control (○) mice. The control animals died *a*, at 215 and *b*, at 230 d of age. The inverse relationship of urine protein and haematocrit is commonly seen in untreated animals. Haematocrits of treated animals have rarely been below 40, or urine protein above 2+. These trends continue.

Figure 2 illustrates the course of two age-matched pairs of animals, treated and untreated, from 3 to 9–10 months of age. Both show the stability of haematocrit and urine protein levels in the Dactinomycin-treated mice, and the inverse relationship of urine protein and haematocrit in the control animals. All untreated animals had heavy proteinuria before death and eight out of 11 had anaemia. Post-mortem renal tissue was evaluated by two observers without knowledge of source, and was judged 3+ or 4+ in severity in all the control animals. Granular staining of immunoglobulin and complement was seen in the glomeruli, with abundant mesangial deposition in most instances and varying amounts of glomerular loop staining. Both the light and fluorescence microscopic findings were typical of our experience in other series.

It seems from this initial group that Dactinomycin alone may suppress B/W disease as well as cyclophosphamide does and probably better than corticosteroids or azathioprine or both. Though a little unkempt in appearance because of loss of hair at injection sites, the animals are in a good condition, with mild or less proteinuria, at well over a year of age, and we plan to continue treating them for their remaining lifetimes.

The majority view of B/W disease today probably includes the following elements: inability to develop or sustain immunological tolerance¹; diminished cell-mediated immunity, following early loss of suppressor cells^{1,4}; and activity of both the Gross and FMR (Friend–Moloney–Rauscher) oncornavirus group^{1,4,12,13}. There has been little evidence that Dactinomycin facilitates tolerance induction¹⁴; in fact, Claman *et al.*¹⁵ found that Dactinomycin administration to mice converted soluble bovine gamma globulin from a tolerogen to an immunogen. In the few studies of cell-mediated immunity in Dactinomycin-treated

animals, responses were neither suppressed nor enhanced^{16,17}. Dactinomycin does have profound effects on the early phase of both Friend and Rauscher leukaemia^{18,19} at the same doses which inhibit normal erythropoiesis, presumably reflecting diminished numbers of erythroid target cells rather than direct effects on the viruses²⁰.

Another possibility is that combination of Dactinomycin with DNA inhibits reactivity with antibody to DNA. There are data suggesting that this is unlikely: Carr *et al.*²¹ developed an assay for human antibody to DNA which employed labelled Dactinomycin–DNA as antigen. They explicitly ruled out any influence by the intercalated Dactinomycin on reaction with antibody.

Although our data indicate that we have suppressed both erythropoiesis and B/W disease by Dactinomycin administration, other mechanisms cannot be ruled out. Immunosuppression, immunopotentiality, anti-viral effects, and interference with antigen–antibody combination have been mentioned, and there may well be others. On the basis of what was known, the profound influence on B/W disease was unexpected, and it suggests that re-examination of the therapeutic potential of Dactinomycin is warranted.

Our mouse colony originated from stocks held at the University of Minnesota, Minneapolis, Minnesota. Merck, Sharp and Dohme donated Dactinomycin. We appreciate the assistance of Miss June Smith, Department of Laboratory Medicine and Pathology, in setting up our facility and training our personnel. We thank Mr Ulrich H. Rudofsky and Dr Rodrigo E. Urizar for reviewing the histology, and Mr Andrew D. Simmons for his assistance with the immunofluorescence microscopy.

ANN E. GABRIELSEN
ALAN S. LUBERT
CAROL T. OLSEN

New York State Kidney Disease Institute,
(Unit of the New York State Department of Health),
120 New Scotland Avenue,
Albany, New York 12208

Received August 3; accepted October 14, 1976.

- Howie, J. B., and Simpson, L. O., in *Lupus Erythematosus* (edit. by Dubois, E. L., 124–141 (University of California Press, Los Angeles, 1974).
- Sommer, V. M., Rudofsky, U. H., and Gabrielsen, A. E., *Clin. exp. Immun.*, **22**, 461–467 (1975).
- Lampert, P. W., and Oldstone, M. B. A., *Science*, **180**, 408–410 (1973).
- Talal, N., and Steinberg, A. D., *Curr. Top. Immun. Microbiol.*, **64**, 79–103 (1974).
- Gabrielsen, A. E., *Lancet*, **ii**, 1116–1118 (1974).
- Windhorst, D. B., Steblay, R. W., Rudofsky, U. H., and Thompson, J. S., *Med. Clin. N.A.*, **54**, 95–105 (1970).
- Tan, E. M., and Kunkel, H. G., *Arth. Rheum.*, **9**, 37–46 (1966).
- Gilliam, J. N., *J. invest. Derm.*, **65**, 154–161 (1975).
- Floersheim, G. L., *Clin. exp. Immun.*, **6**, 861–870 (1970).
- Dixon, W. J., and Massey, F. J., Jr., *Introduction to Statistical Analysis*, 280–286 (McGraw-Hill, New York, 1957).
- Smart, J. V., *Elements of Medical Statistics*, 53–58 (C. C. Thomas, Springfield, Illinois, 1965).
- Yoshiki, T., Mellors, R. C., Strand, M., and August, J. T., *J. exp. Med.*, **140**, 1011–1027 (1974).
- Del Villano, B. C., Croker, B. P., McConeaney, P. J., and Dixon, F. J., *Am. J. Path.*, **82**, 299–314 (1976).
- Gabrielsen, A. E., and Good, R. A., *Adv. Immun.*, **6**, 91–229 (1967).
- Claman, H. N., and Bronsky, E. A., *J. Immun.*, **95**, 718–721 (1965).
- Floersheim, G. L., *Helv. Physiol. Pharmac. Acta*, **22**, 241–256 (1964).
- Floersheim, G. L., *Helv. Physiol. Pharmac. Acta*, **22**, 92–109 (1964).
- Tambourin, P., and Wendling, F., *Nature new Biol.*, **234**, 230–233 (1971).
- Steeves, R. A., Mirand, E. A., and Price, F. W., *Cancer Chemother. Rep.*, **52**, 557–562 (1968).
- Chirigos, M. A., *Rev. Pharmac.*, **9**, 431–456 (1969).
- Carr, R. I., Koffler, D., Agnello, V., and Kunkel, H. G., *Clin. exp. Immun.*, **4**, 527–536 (1969).

Hyperplastic nodules after portacaval anastomosis in rats

FOCAL proliferative or hyperplastic regions of liver cells are readily recognised in human liver diseases^{1,2} and there is a possibility that some types of hyperplastic nodules are important for the development of liver cancer³. The case for the precancerous nature of hyperplastic nodules however, rests entirely on data derived from chemical hepatocarcinogenesis and it is striking that toxic chemicals which are

not known to lead to cancer do not induce hyperplastic nodules, whereas almost all hepatic carcinogens do give rise to nodules⁴. It seems of interest therefore to report the regular development of hyperplastic nodules in rats subjected to portacaval anastomosis without the addition of a known carcinogen to the diet or drinking water.

Male white rats bred in the Sutton Bonington Agricultural Unit, University of Nottingham, were maintained on Diet 41B supplied by Lillico Ltd. They were subjected to portacaval anastomosis (PCA) or sham operation⁵ under ether anaesthesia at 150–200 g and housed six to a cage, on wire grids after the second postoperative day. PCA animals in groups of seven and controls in groups of three were killed after 1 week, 6 weeks, 2, 4, 6, 9 or 12 months. A further six rats were killed at 18 months and six were subjected to partial hepatectomy at 12 months and killed 5–8 months later. Apart from two PCA and one control rat in the first seven groups which were kept for glutaraldehyde perfusion, the liver of each rat was suspended in formal-saline and thin slices were fixed also in formal-alcohol. Dye was injected into the spleens of two controls, two 4-month PCA and three 6-month PCA to check for the presence of a collateral portal flow into the liver⁶.

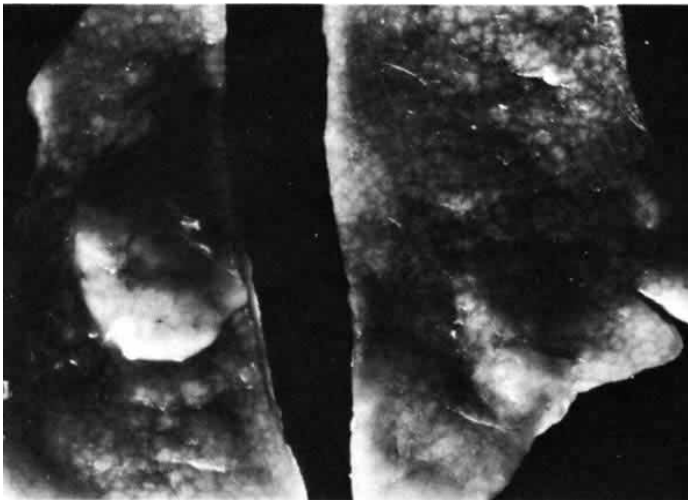


Fig. 1 Hyperplastic tissue extending beyond inferior surfaces of liver 4 months after PCA. Pattern elsewhere regular ($\times 4$).

The animals recovered quickly from the operation and body weight was reduced for about a week, after which the weight gain paralleled that of controls. Liver weights were consistently lower (mean approximately 2% of body weight compared with approximately 3.5% in controls). The anatomical markings were more distinct after 1 week and by 6 weeks the liver surfaces were mottled dark and pale pink. These contrasting markings were accentuated by the second month and although many pale regions were regular but convex by 4 months, confluent foci were found and measured up to 1.0 cm in diameter (Fig. 1). By 6 months such hyperplastic foci varied in size and were found in all PCA animals; in two out of seven animals, masses extended beyond the borders of the median or anterior lobes of the liver. By 12 months, in every instance, the borders and surfaces of the livers were distorted by pale irregular hyperplastic regions, and masses, sometimes polypoid, projected into the peritoneal cavity (Fig. 2). Markedly irregular nodularity was striking after 18 months (Fig. 3).

Microscopy showed that the foci at first consisted of parenchymal cells which contained normally arranged vessels separated by atrophied cells in the perivenous regions, with evidence of compression by 6 months and loss of normal vascular relationships at 12 and 18 months (Fig. 4). Foci of crowded cells were noted, but no unequivocal malignant transformation was detected. Early

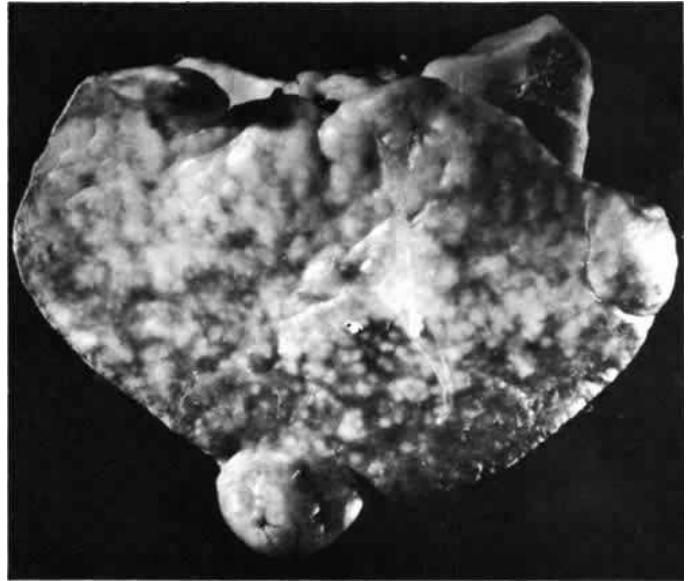


Fig. 2 Much distortion of liver surface by hyperplastic tissue, with nodule on inferior border of median lobe. Pattern elsewhere irregular. 12 months after PCA ($\times 2.3$).

changes were found in all experimental animals but not in controls and later changes with obvious distortion were noted to a lesser or greater extent in all PCA animals. Although adhesions were found quite frequently, portal revascularisation was not demonstrated on dye injection. The changes were more marked in animals subjected to partial hepatectomy in addition to portacaval anastomosis.

These experiments form part of an investigation into the reactions of atrophied hepatocytes. Such atrophied cells are known to undergo hyperplasia after the sudden reduction of hepatic tissue effected by partial resection in the PCA rat⁷. It therefore seemed possible that the rather slower, but nonetheless substantial, loss of parenchyma inherent in the response to the uncomplicated PCA also might effect a stimulus to hyperplasia. Such hyperplasia as occurred in this series seemed to involve at first predominantly periportal cells and it is not clear what part such anatomical

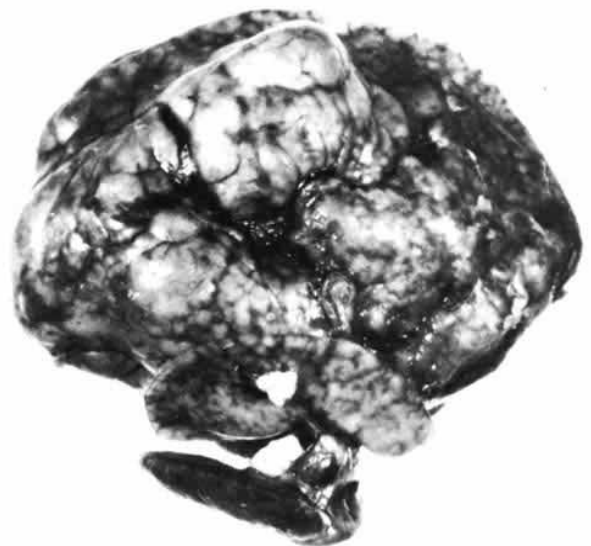


Fig. 3 Large nodule formation in liver remnant 18 months after PCA and 6 months after partial hepatectomy ($\times 2.3$).

zonation might play in the ultimate development of hyperplastic nodules. The appearance of the hyperplastic nodules is similar to those induced by chemical carcinogens, but it is not yet known whether similar changes in biochemical properties and the antigenic complement of the hepatocytes occur^{3,4}. It would clearly be of major interest to ascertain whether a preneoplastic antigen is detectable in these circumstances⁵.

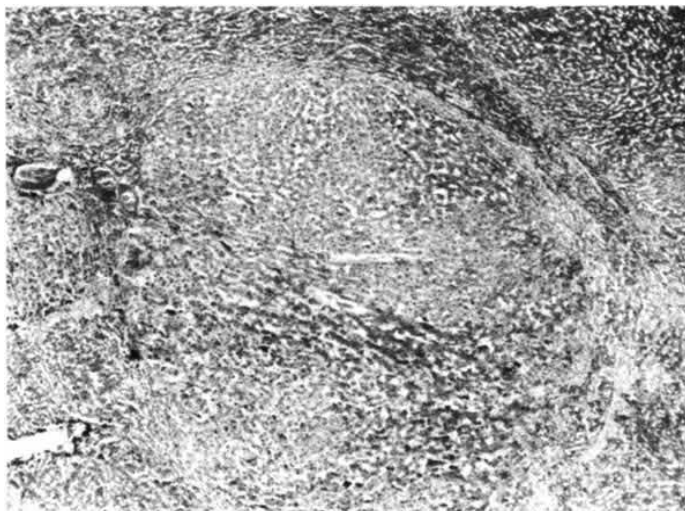


Fig. 4 Irregular hyperplastic nodule with compression of adjacent parenchyma and distortion of hepatic plates, taken from liver shown in Fig. 3. Haematoxylin and eosin ($\times 33.6$).

It is puzzling that these changes have not been mentioned previously^{6,8-10}. Most papers report that the microscopic appearances of livers from PCA rats are remarkably normal and that it is difficult to distinguish these from control livers. Microscopy of the present series confirms this superficial impression in early cases and, for the most part, vascular relationships are retained and plates of hepatocytes are flanked by sinusoids with a good complement of littoral cells^{6,9}. It is only when a comparison is made with macroscopic appearances that the irregularity, which is much more striking macroscopically and sometimes difficult to define microscopically particularly before 6 months, can be identified. After longer periods the compression of these foci on adjacent parenchyma is clearer and eventually the vascular pattern is much distorted, but this is a late phenomenon. At this stage, microscopy is revealing but only if the relevant part is selected. It seems possible therefore, that the changes described may easily not be noted unless gross examination is combined with microscopy.

Other possibilities include strain differences in the rats, housing and dietary effects. Strain differences are difficult to evaluate but in common with most other colonies none of our controls showed evidence of a tendency to nodule formation and at least one report refers to a similar strain⁶. Animal housing is relevant to the well-being of the experimental animals¹¹ and wire-bottomed cages much improve the clinical state of the rats, but there is no suggestion in this or any other work that this form of caging is conducive to nodule formation. It is difficult to see how the reduction in coprophagy might effect an increased rate of DNA synthesis, by pathways involving a reduced B_{12} metabolism or any others. With regard to the possibility of dietary effects, it is clear that PCA animals in general have a lower dietary intake and the animals in this series were no exception. The rate of increase in body weight was, however, comparable with that of controls and similar to that described for all other series. Pair feeding was not an effective procedure in a long term experiment involving wire-bottomed cages, because substantial quantities of food were noted in the waste and accurate quantitative recovery was

not carried out. There were no conventional stigmata of dietary deficiency on clinical or microscopical examination and the incidence of intercurrent disease was at the same rate in experimental and control rats, occurring for the most part in old animals.

We thank the Medical Research Council for a project grant awarded to K.W. and Miss Yvonne Maddison for technical help.

K. WEINBREN

S. L. A. WASHINGTON

Department of Histopathology,
Royal Postgraduate Medical School,
DuCane Road,
London W12 0HS, UK

Received August 19; accepted October 5, 1976.

- ¹ Steiner, P. E., *Am. J. Path.*, **35**, 943-953 (1959).
- ² Blendis, L. M., Parkinson, M. C., Shilkin, K. B., and Williams, R., *Q. Jl. Med.*, **43**, 25-32 (1974).
- ³ Okita, K., and Farber, E., *GANN Monogr. Cancer Res.*, **17**, 283-299 (1975).
- ⁴ Farber, E., *Methods in Cancer Research*, 7 (edit. by Busch, H.), 345-375 (Academic, London, 1973).
- ⁵ Weinbren, K., Washington, S. L. A., and Smith, C. Y., *Br. J. exp. Path.*, **56**, 148-156 (1975).
- ⁶ Kyu, M. H., and Cavanagh, J. B., *Br. J. exp. Path.*, **51**, 217-227 (1970).
- ⁷ Fisher, B., Lee, S. H., Fisher, E. R., and Saffer, E., *Surgery*, **521** 88-102 (1962).
- ⁸ Assal, J.-Ph., Levrat, R., Cahn, T., and Renold, A. E., *Z. ges. exp. Med.*, **154**, 87-100 (1971).
- ⁹ Lee, S., Chandler, J. G., Broelsch, C. E., Flamant, Y. M., and Orloff, M. J., *J. surg. Res.*, **17**, 53-73 (1974).
- ¹⁰ Meyers, O. L., Hickman, R., Kerraan, M., Engelbrecht, G. H. C., Webber, B. L., and Terblanche, J., *S. Afr. med. J.*, **49**, 1048 (1975).
- ¹¹ Herz, R., Sautter, V., Robert, F., and Bircher, J., *Eur. J. clin. Invest.*, **2**, 390-397 (1972).

Induction of oncogenic transformation *in vitro* by ultraviolet light

SKIN fibroblasts derived from patients with the autosomal recessive disease *xeroderma pigmentosum* are defective in excision repair of photodamage induced by short wavelength ultraviolet light¹. In conjunction with the clinical observation that these patients are extremely prone to develop skin cancer², it has been suggested that DNA repair plays a central role in the process of carcinogenesis. Although methodologies are available for investigating the molecular biology of mammalian DNA repair, studies of carcinogenesis induced by UVL have been limited largely to the use of the hairless mouse³⁻⁵. In a preliminary report, DiPaolo and Donovan⁶ reported the induction of transformation in primary hamster embryo cell cultures by ultraviolet light. Mondal and Heidelberger⁷ reported inducing malignant transformation in mouse 10T1/2 cells *in vitro* by exposure to ultraviolet light followed by treatment with tetradecanoyl phorbol acetate. We report here that ultraviolet light of 254-nm wavelength alone is sufficient to induce reproducible transformation in this cell line, and we present the dose-response relationship for this phenomenon.

We used the C3H mouse-embryo-derived cell line designated 10T1/2 clone 8 (ref. 8). Stock cultures were carried in large glass bottles in Eagle's basal medium supplemented with 10% heat-inactivated foetal calf serum (Gibco) and antibiotics. Cells were in passages 11-15. Transformation was scored as previously described^{9,10}. Only type III foci were scored as transformants: these foci grow in a multi-layer, criss-crossed fashion over a background monolayer of normal cells, and exhibit a highly polar, fibroblastic morphology. When injected into syngeneic mice, they cause fibrosarcomas^{10,11} while normal cells do not. The transformed foci we have obtained with ultraviolet light do not differ in morphology from those obtained with X-ray⁹ or

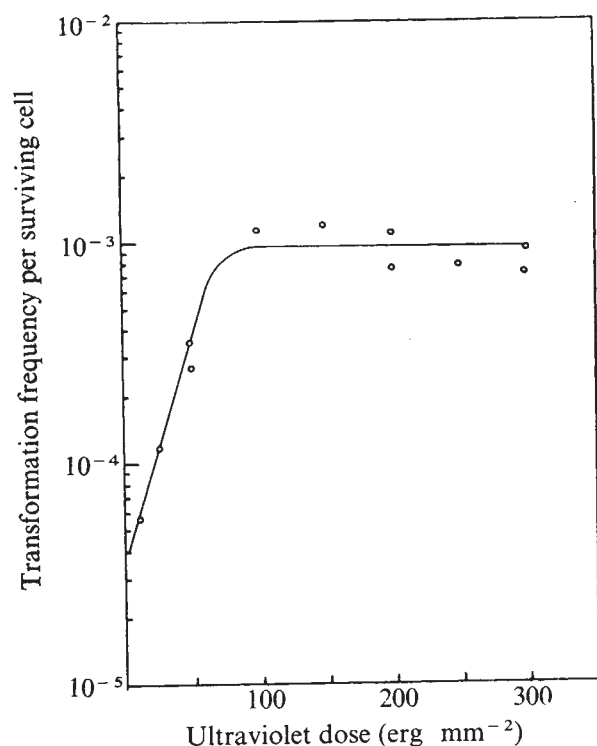


Fig. 1 Transformation frequency per surviving cell as a function of UVL dose for the 10T1/2 mouse fibroblast cell line. 100-mm plastic dishes (Falcon) were seeded with 300–400 surviving cells each to allow them to develop approximately 14 doublings before confluence was reached⁹. The medium was removed 20 h after plating and the cells were irradiated by a bank of five GE G8T5 tubes emitting predominantly 254 nm ultraviolet light at a dose rate of $4.5 \text{ erg mm}^{-2} \text{ s}^{-1}$ at the cell surface. After irradiation, cells were overlaid with fresh medium and returned to a 5% CO_2 humidified incubator held at 37°C . The medium was then changed twice weekly until confluence, then weekly until the seventh week, at which time the cells were fixed with Bouin's solution, stained with Trypan blue and scored for transformants. For each data point, 20–60 transformation plates were used while four plates, each seeded with a cell density one-fifth that of the transformation plates, were terminated at the end of the 2nd week to assay for the number of cells surviving each treatment.

chemical carcinogens^{10,12}. No type III transformants were seen in unirradiated control cultures in any of these experiments.

Figure 1 presents the dose-response curve for ultraviolet-induced transformation. The transformation frequency on the ordinate is plotted as transformants per surviving cell. Clearly this transformation frequency rises initially at low doses, but reaches a plateau after an inflection point at about 75 erg mm^{-2} . The existence of a plateau implies that over this higher dose range a constant proportion of the surviving cells (as assayed by the ability to proliferate indefinitely) is transformed. Thus, when the results are expressed as transformants per irradiated cell (Fig. 2, lower curve), the portion of the curve which corresponds to the plateau is parallel to that part of the dose-response curve for survival (Fig. 2, upper curve). In accordance with our assertion that both survival and transformation are cell-mediated processes¹¹, these data are consistent with the hypothesis that beyond a limiting amount of damage, the relative efficiencies of the cellular repair mechanisms which mediate survival and transformation remain constant. Transformation is genetically selected neither for nor against.

The plateau region in Fig. 1 spans a dose range occupied by both components of the biphasic survival curve (Fig. 2, upper curve). The second component has been shown to represent a subpopulation consisting predominantly of cells

in the G_2 phase of the cell cycle¹³. Host-cell reactivation studies of ultraviolet-irradiated herpes-virus¹⁴ have suggested that the superior survival characteristics of this subpopulation, as evidenced by a greater than twofold increase in the D_0 of the survival curve, can at least in part be attributed to additional repair processes that are operational only in these cells. Our data suggest that no selective advantage has been conferred on this subpopulation with respect to transformation, raising the possibility that the additional repair responsible for the second component of the survival curve represents increased activity of existing processes rather than the operation of a new process. For example, the operation of a more or less error-prone repair mechanism does not seem to be involved.

At the low dose region, although there is an initial shoulder on the survival curve, the curve for transformation per irradiated cell (Fig. 2, lower curve) has an initial

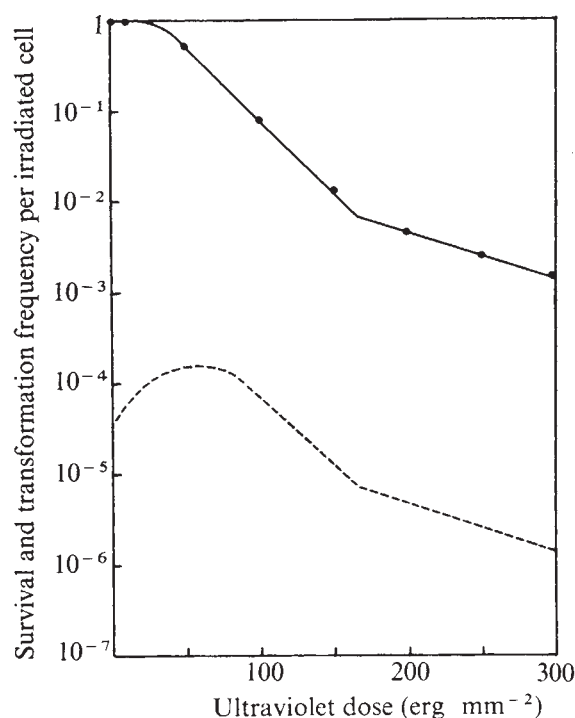


Fig. 2 Dose-response curve for survival (●) and transformation frequency (—) per irradiated cell.

positive slope, at least at doses greater than 10 erg mm^{-2} . It is the combination of the two processes of transformation and cell killing that produces the initial rise and the subsequent decline of this curve. These results are thus qualitatively similar to those for X-ray-induced transformation^{9,15} and other experimental evidence for the existence of a most efficient dose for radiation-induced cancer.

We propose that the transformation seen in these experiments results from the operation of the postreplication repair mechanism which is thought to be error prone. Excision of pyrimidine dimers in the 10T1/2 cell line has been shown to be below the level of detection (personal communication from J. S. Bertram). Moreover, since the cells in our experiments were actively traversing the cell cycle at the time of exposure to ultraviolet light, one can envisage that the presence of unexcised dimers at the first postirradiation DNA synthesis would necessitate the operation of the postreplication repair mechanism. The operation of this mechanism would introduce errors into the genome, some of which would lead ultimately to the fixation of transformation^{16–18}.

Our results suggest that established cell lines such as the 10T1/2 mouse fibroblast might be used to study quantitatively ultraviolet-induced malignant transformation *in vitro*, and in particular to investigate the role of ultraviolet-induced DNA repair processes with respect to the end point of transformation. Such experiments are in progress.

This work was supported by grants from the NCI and NIEHS.

GERALD L. CHAN
JOHN B. LITTLE

Laboratory of Radiobiology,
Department of Physiology,
Harvard University,
School of Public Health,
Boston, Massachusetts 02115

Received August 9; accepted September 27, 1976.

- ¹ Cleaver, J. E., *Nature*, **218**, 652-656 (1968).
- ² Robbins, J. H., Kraemer, K. H., Lutzner, M. A., Festoff, B. W., and Coon, H. G., *Ann. Int. Med.*, **80**, 221-248 (1974).
- ³ Chopra, D. P., *J. invest. Dermatol.*, **66**, 242-247 (1976).
- ⁴ Hsu, J., Forbes, P. D., Harber, L. C., and Lakow, E., *Photochem. Photobiol.*, **21**, 185-193 (1975).
- ⁵ Zajdela, F., and Latarjet, R., *C. r. hebdo. Séanc., Acad. Sci. (Paris)*, Ser. D, **277**, 1073-1076 (1973).
- ⁶ DiPaolo, J. A., and Donovan, P. J., *Int. J. radiat. Biol.* (in the press).
- ⁷ Mondal, S., and Heidelberger, C., *Nature*, **260**, 710-711 (1976).
- ⁸ Reznikoff, C. A., Brankow, D. W., and Heidelberger, C., *Cancer Res.*, **33**, 3231-3238 (1973).
- ⁹ Terzaghi, M., and Little, J. B., *Cancer Res.*, **36**, 1367-1374 (1976).
- ¹⁰ Reznikoff, C. A., Bertram, J. S., Brankow, D. W., and Heidelberger, C., *Cancer Res.*, **33**, 3239-3249 (1973).
- ¹¹ Terzaghi, M., and Little, J. B., *Nature*, **253**, 548-549 (1975).
- ¹² Benedict, W. F., *Nature*, **260**, 368-369 (1976).
- ¹³ Williams, J. R., thesis, Harvard Univ. (1972).
- ¹⁴ Lytle, C. D., *Int. J. Radiat. Biol.*, **22**, 167-174 (1972).
- ¹⁵ Borek, C., and Hall, E., *Nature*, **243**, 450-453 (1973).
- ¹⁶ Kakunaga, T., *Nature*, **258**, 248-250 (1975).
- ¹⁷ Kondo, S., in *Advances in Biophysics*, 7 (edit. by Kotani, M.), 91-162 (University of Tokyo Press, 1975).
- ¹⁸ Witkin, E. M., in *Molecular Mechanisms for Repair of DNA* (edit. by Hanawalt, P., and Setlow, R. B.), Part A, 347-355 (Plenum, New York, 1975).
- ¹⁹ Hanawalt, P., in *Molecular Mechanisms for Repair of DNA* (edit. by Hanawalt, P. G., and Setlow, R. B.), Part B, 421-430 (Plenum, New York, 1975).

Asbestos cytotoxicity in a long term macrophage-like cell culture

ASBESTOS is well documented as a carcinogen and cocarcinogen in man and experimental animals, and malignancy may appear after exposure to small amounts. In people who have worked with asbestos insulation there has often been a lag of 20 yr between the first exposure and the development of neoplasia. Among these workers a high incidence of carcinoma of the lung and a high frequency of the rare neoplasm mesothelioma has been reported¹. The risk of death from lung cancer is 92 times greater for an asbestos worker who smokes than for someone who is statistically comparable and neither smokes nor works with asbestos. The additive effect of smoking and exposure to asbestos is much greater than that of either factor alone, indicating a cocarcinogenic effect². Exposure to asbestos is not confined to the occupational environment, for asbestos is present in the air of 49 cities in the USA, in some talcum powders and gypsum spackles and in about 400 industrial products³. As the initial *in vivo* response to asbestos involves uptake by host macrophages⁴, we have quantified the effects of standard Union Internationale contre le Cancer (UICC) reference samples of amosite and chrysotile B asbestos *in vitro* on the P388D1 line of malignant cells. These cells have macrophage-like characteristics⁵, but, unlike fresh culture systems, they are capable of reproduction and make long term experimentation possible. We found that asbestos toxicity could be measured as a function of the percentage of cells associated with fibres. Chrysotile was more toxic per unit weight than amosite because it had more fibres per unit weight that could become associated with cells.

Available information on the basic biological effects of asbestos in macrophage cell culture systems has been

reviewed recently⁶. In previous studies fresh peritoneal cultures of hamster, mouse and guinea pig macrophages were exposed to samples of amosite and chrysotile⁷⁻¹². The resulting cytotoxicity was thought to be due to the interaction of asbestos fibres with plasma membrane, and was lessened by increasing the amount of serum. A delayed cytotoxicity was demonstrated by the release of increased amounts of acid phosphatase and β -glucuronidase into the media. In all these cases chrysotile was more toxic per unit weight than amosite.

The crystalline properties of asbestos enabled us to use rectified polarisation optics to detect small birefringent particles of asbestos fibres associated with cells in culture. We used UICC standard reference samples of amosite and chrysotile B, which have been characterised extensively¹³⁻¹⁵. The external surface area of the amosite is $3.3 \text{ m}^2 \text{ g}^{-1}$ while that of the chrysotile is $4.9 \text{ m}^2 \text{ g}^{-1}$. In the optical microscope fibres of amosite and chrysotile vary from 2 to 60 μm long. Chrysotile has 6% more fibres $>4 \mu\text{m}$ than amosite. Previous studies have shown that mouse peritoneal macrophages will phagocytose completely asbestos fibres less than 5 μm long. Fibres between 5 and 20 μm long are sometimes ingested completely while beyond this length complete phagocytosis is not possible.

With both amosite and chrysotile asbestos we found dose-response cytotoxicity. All cultures which reached a nadir of

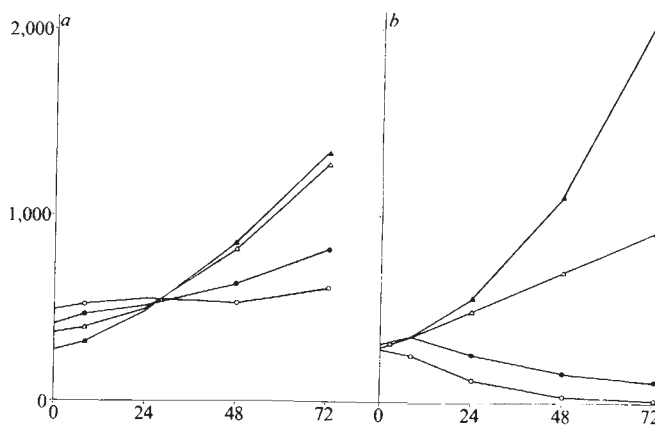


Fig. 1 Growth curves showing the number of P388D1 cells after exposure to amosite (a) and chrysotile (b). Four 250-ml Falcon tissue culture flasks were each inoculated with 10 ml of 4×10^5 viable P388D1 cells per ml of passage number 37 for the amosite and passage number 41 for the chrysotile. Fischer's media for leukaemic cells of mice plus 10% foetal calf serum with a total volume of 10 ml per flask was used. The flasks were incubated stationary at 37 °C in 5% CO_2 in air. After 24 h the medium was changed in all flasks and steam-sterilised asbestos was added to three of the flasks in concentrations of 10, 50 and 100 $\mu\text{g ml}^{-1}$ with a total volume of 10 ml per flask. The fourth flask was used as a normal uninoculated control. Medium containing asbestos was left in contact with the cells for 48 h and then removed. Throughout the experiment medium without asbestos was used for complete changes of medium on a regular basis, generally every 2 d. To standardise observations of the culture fields, paper tape with 1-mm holes was taped randomly to the outside of the flasks. There were 10 observation holes for each flask of cells. Using an inverted Nikon microscope fitted with bright field phase objectives and $\times 10$ eyepieces, each field was first focused and observed with a $\times 10$ objective to maintain uniformity of field position, and photographs were then taken through the $\times 20$ objective. This allowed repeated photographs of the same field of cells. Observations and photographs were made immediately before (time zero) the addition of asbestos-containing medium, and then after 8, 24, 48 and 72 h. Each of the 10 fields on each flask was photographed at each time point, and the total of morphologically identifiable cells per photograph was counted in a standardised manner by one observer. Growth curves were derived from these counts. ▲, normal; △, asbestos (10 $\mu\text{g ml}^{-1}$); ●, 50 $\mu\text{g ml}^{-1}$; ○, 100 $\mu\text{g ml}^{-1}$. At all doses the standard error of the mean (s.e.m.) was less than 15%, except for chrysotile at 50 $\mu\text{g ml}^{-1}$ and 100 $\mu\text{g ml}^{-1}$ at 48 and 72 h, when it was 33%.

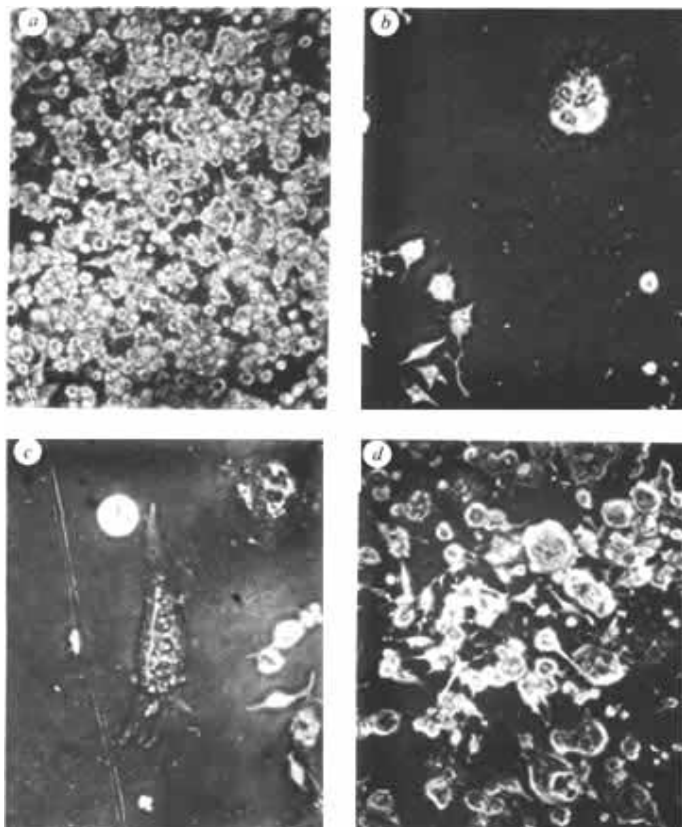


Fig. 2 Morphology of the P388D1 cells after 8–10 d exposure to UICC samples of amosite and chrysotile B asbestos ($50 \mu\text{g ml}^{-1}$). *a*, Normal control after 10 d. Note the large number of small round cells. *b*, After 10 d of exposure to chrysotile ($50 \mu\text{g ml}^{-1}$). Cells are scarce and have changed in size, accompanied by the 'sunburst' morphology. *c*, After 8 d of exposure to chrysotile ($50 \mu\text{g ml}^{-1}$). Note the later conformation of this cell to the shape of the cell-associated fibre. *d*, After 10 d of exposure to amosite ($50 \mu\text{g ml}^{-1}$). The number of cells present and their size morphology should be compared with those of the chrysotile (Fig. 2). ($\times 200$).

growth 72 h after exposure to asbestos maintained the ability to become confluent again within a time proportional to the initial decrease in cell number. Morphological changes occurred after the initial exposure to asbestos. With amosite these included increasing size, numerous thin dendritic projections and an initial change of configuration of a cell conforming to the shape and size of the particles with which the cell was associated. In contrast, although cells exposed to chrysotile did not appear to change shape initially to conform with that of the particles, they often did so later. They soon became larger than normal cells or those exposed to amosite, and took on a characteristic surface appearance with numerous short projections in a "sunburst" pattern.

Table 1 Effects of percentage of cell-associated fibres after 3 h on 72-h cell growth

Asbestos	Dilution ($\mu\text{g ml}^{-1}$)	Time (h)	% of cell-associated fibres (coverslips)	% of cell death at 72 h (bottles)
Amosite	10	3	36.9	4.7
Amosite	50	3	100	38.6
Amosite	100	3	100*	54.0
Chrysotile	10	3	96.4	55.0
Chrysotile	50	3	100†	94.3
Chrysotile	100	3	—‡	99.1

*More than one fibre per cell.

†Three or four fibres per cell.

‡A few cells left.

Untreated P388D1 cells had little birefringence except that of the mitotic spindle. Toxicity of both amosite and chrysotile was related to the percentage of cells with associated birefringent asbestos fibres (Table 1). For example, the similar percentage of cell-associated fibres present after 3 h with amosite at $50 \mu\text{g ml}^{-1}$ and chrysotile at $10 \mu\text{g ml}^{-1}$ correlated with similar percentages of cell death at 72 h. Asbestos fibres in the cultures were counted from photographs taken after 8 h of exposure: there were 378 fibres with the chrysotile, and 366 fibres with the amosite (concentrations as above). Therefore the difference in toxicity per unit weight of chrysotile and amosite relates to the number of fibres associated per P388D1 cell.



Fig. 3 Birefringence of P388D1 cells after 8 h of exposure to chrysotile ($100 \mu\text{g ml}^{-1}$). Coverslips (25 mm in diameter) in 2 ml of medium were inoculated with the same cells and the same concentrations of both types of asbestos. Observations and photographs were made with a Leitz polarisation microscope fitted with Nikon rectified objectives. Under a polarisation microscope the crystalline nature of asbestos produced increased the contrast of black or white fibres against a grey background. The cell-associated fibres changed contrast at different compensator settings, indicating that the fibres were birefringent. A and P indicate vibration planes of analyser and polariser, respectively. This photograph was taken at a compensator setting 7° to the side of the background compensation position. Measurements were made of the percentage of cells with associated birefringent asbestos fibres at 3 and 12 h after addition of asbestos.

In our quantitative investigation of cytotoxicity we found asbestos to be as cytotoxic for malignant P388D1 cells as it has been reported to be for non-malignant fresh macrophage culture systems. Therefore these malignant cells would make a good model system for the study of long term effects of asbestos-cell interactions. This *in vitro* system should make it possible to gain some insight into the long *in vivo* macrophage effect in the interim between asbestos exposure and the development of neoplasia.

We thank Miss Jo Abbott for assistance in the preparation of the figures.

MARTA J. WADE
LEWIS E. LIPKIN

Image Processing Unit,
Division of Cancer Biology and Diagnosis,
National Cancer Institute

ROBERT W. TUCKER

Oncogenesis and Cell Physiology Section,
Laboratory of Biochemistry,
National Cancer Institute,
National Institutes of Health,
Bethesda, Maryland 20014

ARTHUR L. FRANK

Department of Medicine and
Division of Environmental Medicine,
Mount Sinai School of Medicine,
New York, New York 10029

Received April 19; accepted August 20, 1976.

- ¹ Selikoff, I. J., Churg, J., and Hammond, E. C., *J. Am. med. Ass.*, **188**, 22–26 (1964).
- ² Selikoff, I. J., Hammond, E. C., and Churg, J., *J. Am. med. Ass.*, **204**, 106–112 (1968).
- ³ Selikoff, I. J., et al. in *Biological Effects of Asbestos* (edit. by Rall, D.), 30–31 (Environmental Sciences Laboratory, Mt. Sinai School of Medicine, New York, 1973).
- ⁴ Davis, J. M. G., *Br. J. exp. Path.*, **48**, 379–385 (1967).
- ⁵ Koren, H. S., Handwerker, B. S., and Wunderlich, J. R., *J. Immun.*, **114**, 894–897 (1975).
- ⁶ Harrington, J. S., Allison, A. C., and Badami, D. V., *Adv. Pharm. Chemother.*, **12**, 291–402 (1975).
- ⁷ Allison, A. C., *Arch. int. Med.*, **128**, 131–139 (1971).
- ⁸ Allison, A. C., in *Cell Interactions*, third Lepetit Colloquium (edit. by Silvestri, L. G.), 156 (North-Holland, Amsterdam, 1972).
- ⁹ Bey, E., and Harrington, J. S., *J. exp. Med.*, **133**, 1149–1169 (1971).
- ¹⁰ Miller, K., and Harrington, J. S., *Br. J. exp. Path.*, **53**, 397–405 (1972).
- ¹¹ Robock, K., and Klosterkotter, W., in *Inhaled Particles 111* (edit. by Walton, W. H.), 453–465 (Unwin, London, 1971).
- ¹² Robock, K., and Klosterkotter, W., in *Fourth Int. Pneumoconiosis Conf.* (edit. by Int. Labour Organisation), 2–10 (Ministry of Labour, Bucharest, 1971).
- ¹³ Rendall, R. E. G., in *Pneumoconiosis*, Proc. Int. Conf., Johannesburg, 1969 (edit. by Shapiro, H. A.), 23–27 (Oxford University Press, Cape Town, 1970).
- ¹⁴ Timbrell, V., in *Pneumoconiosis*, Proc. Int. Conf., Johannesburg, 1969 (edit. by Shapiro, H. A.), 28–36 (Oxford University Press, Cape Town, 1970).
- ¹⁵ Timbrell, V., and Rendall, R. E. G., *Powder Tech.*, **5**, 279–287 (1971–1972).

Effect of a phorbol ester on a transformation-sensitive surface protein of chick fibroblasts

PMA (phorbol myristate acetate) is the most active of a class of compounds present in croton oil which act as tumour promoters^{1–3}. While PMA possesses little carcinogenicity in its own right, its repeated application to mouse skin causes tumour induction in animals treated up to several months previously with a single subthreshold dose of a carcinogen. If the order of treatments is reversed, that is, if PMA treatment precedes that with the carcinogen, then no tumours are induced. Studies both with mouse skin and with cells in tissue culture indicate substantial effects of PMA on cell behaviour and biochemistry. Many of these effects such as alteration of phospholipid synthesis⁴ or stimulation of Na⁺, K⁺-ATPase and 5'-nucleotidase⁵ relate to the cell membrane. We report here that PMA alters the cell surface protein composition of chick embryo fibroblasts, causing a decrease in the major surface protein LETS (molecular weight 250,000). Previous work by several laboratories has indicated that LETS, which is present on the surface of fibroblasts from a variety of species, either decreases or disappears upon transformation^{6–11}. Although LETS is highly sensitive to cleavage by proteases such as plasmin¹², its mechanism of loss following transformation remains to be clarified.

We find that the addition of PMA to exponentially growing cultures of secondary chick embryo fibroblasts led to alterations in the pattern of iodinated surface proteins fractionated on sodium dodecyl sulphate (SDS)-polyacrylamide gels. Several bands were decreased in amount,

while at least one new band appeared. Most striking was a reduction in the amount of LETS. After incubation of exponentially growing chick embryo fibroblasts in the presence of PMA for 3 d, the amount of radioactivity in LETS per cell was decreased to 12% of the control value (Fig. 1). The magnitude of the decrease in LETS depends on the basis used for comparison. PMA had little effect under the usual experimental conditions on cell number per plate,

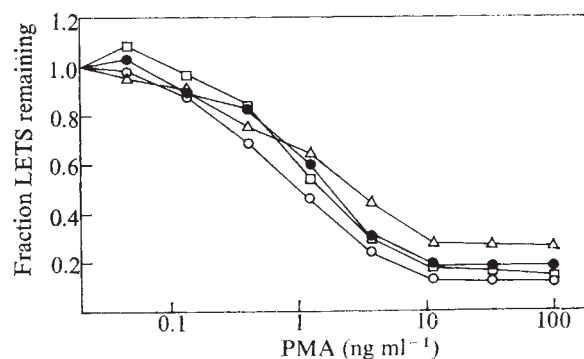


Fig. 1 Effect of PMA on LETS. Secondary chick embryo fibroblasts were plated at 1×10^5 per 60 mm dish in MEM containing 10% tryptose phosphate broth, 5% heat-inactivated chicken serum, penicillin (75 u ml^{-1}) and streptomycin (50 µg ml^{-1}). PMA (Consolidated Midland) dissolved in DMSO was added to each plate to give the indicated final concentrations. The concentration of DMSO in all plates was constant, 0.10%. After 3 d, the cells were iodinated, the proteins fractionated by SDS-polyacrylamide gel electrophoresis on 5% gels, and the amounts of LETS determined as described¹². The "fraction LETS remaining" gives the c.p.m. in LETS from the plate with PMA per c.p.m. in LETS from the control plate with DMSO. The data were normalised where indicated as follows: (○) Fraction remaining; (□) Fraction remaining, normalised to cell number; (●) Fraction remaining, normalised to the total surface label migrating on the SDS-polyacrylamide gels with mobility between that of LETS and the marker dye; (△) Fraction remaining, normalised to total protein. All points are the average of duplicate measurements. 1.0 = 31,000 c.p.m. in LETS/gel sample.

total iodine label incorporated, or incorporated iodine label migrating on SDS-polyacrylamide gels with a mobility between that of LETS and of the marker dye bromophenol blue. The decrease in the amount of LETS relative to that of controls was thus similar when normalised to any of these bases. While the amount of LETS relative to other surface label is probably the most relevant value, the volume of chick embryo fibroblasts treated with PMA decreased by a factor of two (manuscript in preparation) and the amount of protein per cell decreased comparably. Consequently, the amount of LETS was reduced to 25% of that in the untreated control when expressed per µg of cell protein.

The growth state of cells in culture is often a significant variable affecting cell surface properties. The observed decrease in LETS caused by PMA probably cannot be explained by such an effect. In the conditions of Fig. 1, the addition of PMA had little effect ($\pm 20\%$) on absolute cell number per plate, on the rate of increase in cell number, and on incorporation of ³H-thymidine per cell.

The amount of PMA required for the half-maximal effect on LETS was $1\text{--}2 \text{ ng ml}^{-1}$. This value corresponds to a concentration of $1.5\text{--}3 \times 10^{-9} \text{ M}$. Increase in the concentration of PMA between 10 and 100 ng ml^{-1} had no further effect on LETS.

The decrease in LETS following addition of PMA proceeded over a 3-d time course. If cells were replated at this time into fresh PMA-containing medium, this low level was maintained. This loss was slower than that reported after transformation of chick embryo fibroblasts by Rous sarcoma virus⁸. Likewise, full expression of the effects of

PMA on LETS took much longer than its effects on some other properties, for example stimulation of ^3H -deoxyglucose uptake in chick embryo fibroblasts (4 h) or stimulation of cyclic GMP in 3T3 cells (1 min)¹³. It should be noted that the kinetics of loss of LETS after addition of PMA refer to the total amount of LETS remaining on the cell surface. The rapidity of the response to addition of PMA in the production and/or attachment of new LETS remains to be established.

The loss of LETS brought about by PMA was not an irreversible process. Cells treated with PMA regained LETS within 3 d of trypsinisation and replating in medium lacking PMA. PMA was thus not inducing permanent transformation of the cells. The 3-d time course for reappearance of LETS represents an upper limit on the recovery time. Since PMA is highly lipid soluble¹⁴, the possible role of residual cell-bound PMA in delaying recovery of LETS is not known. For comparison, upon shift to the non-permissive temperature of chick embryo fibroblasts transformed by a temperature sensitive mutant of Rous sarcoma virus, LETS returned to normal levels with a 1-d time course⁵.

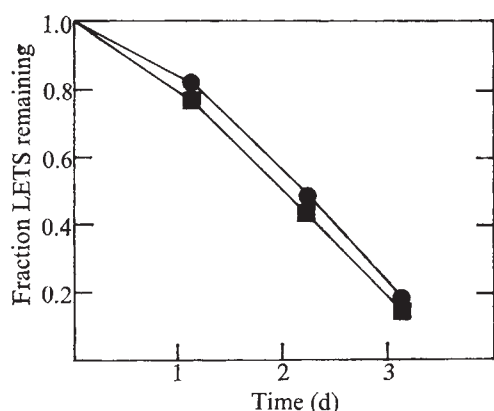


Fig. 2 Time course of decrease in LETS after addition of PMA. Cells were plated and treated with PMA as described in the legend to Fig. 1, except that the initial plating densities were varied so that the cell densities at the time of iodination were similar. At the indicated times, plates treated with DMSO or PMA were iodinated and LETS determined. Fraction LETS remaining has been normalised to the total surface label migrating on the SDS-polyacrylamide gel with a mobility between that of LETS and the marker dye. (●) PMA, 3 ng ml⁻¹. (■) PMA, 10 ng ml⁻¹.

The direct cause for the decrease in LETS upon treatment of the chick embryo fibroblasts with PMA is not known. For the loss of LETS induced by viral transformation, the following possibilities have been considered: (1) failure of the cells to synthesise LETS, (2) failure of LETS to be properly inserted and bound to the membrane, or (3) proteolytic cleavage of LETS from the membrane after binding. With regard to an earlier level of regulation, the possibility that PMA does not affect LETS *per se* but regulates some intermediate property, which itself determines the level of LETS, deserves consideration. In either case, the understanding of the mode of action of PMA on LETS is of particular interest in that it provides a new approach and may potentially provide new insight into the control of this same surface component by Rous sarcoma virus.

A number of the effects of PMA on cells resemble those induced by transformation. These effects include (1) decrease in LETS, (2) increase in plasminogen activator¹⁵, (3) release from contact inhibition¹⁶, (4) increase in cyclic GMP¹³ and decrease in cyclic AMP concentrations¹⁷, and (5) reduction in cell adhesion, loss of orientation on the

plate and alteration in morphology¹⁸. Caution must be exercised in extrapolating these results since a number of these changes have been observed in systems other than those, for example the mouse epidermis and certain cell culture systems¹⁹, where the promoting effects of PMA have been characterised. Nonetheless, the parallels between the effects on cells of PMA and of transformation are consistent with the hypothesis that tumour promoters might function by causing the conversion of cells from a normal phenotype to one more resembling the transformed state, thus facilitating expression of the genotype of latent tumour cells.

This research was supported by a grant from the Milton Fund and by a grant from the National Institutes of Health. P.M.B. is recipient of an Andrew W. Mellow award; P.E.D. is a National Science Foundation predoctoral fellow; P.W.R. is a postdoctoral fellow of the Cystic Fibrosis Foundation. S. Khong provided technical assistance.

PETER M. BLUMBERG
PAUL E. DRIEDGER
PETER W. ROSSOW

Department of Pharmacology,
Harvard Medical School,
Boston, Massachusetts 02115

Received July 12; accepted October 12, 1976.

- 1 Berenblum, I., in *Cancer*, 1 (edit. by Becker, F. F.), 323-344 (Plenum, New York, 1975).
- 2 Hecker, E., *Meth. Cancer Res.*, 6, 439-484 (1971).
- 3 Van Duuren, B. L., *Prog. exp. Tumor Res.*, 11, 31-68 (1969).
- 4 Rohrschneider, L. R., and Boutwell, R. K., *Cancer Res.*, 33, 1945-1952 (1973).
- 5 Sivak, A., Mossman, B. T., and Van Duuren, B. L., *Biochem. biophys. Res. Commun.*, 46, 605-609 (1972).
- 6 Robbins, P. W., et al., *Cold Spring Harb. Symp. quant. Biol.*, 39, 1173-1180 (1974).
- 7 Hynes, R. O., *Proc. natn. Acad. Sci. U.S.A.*, 70, 3170-3174 (1973).
- 8 Hynes, R. O., and Wyke, J. A., *Virology*, 64, 492-504 (1975).
- 9 Vaheri, A., and Ruoslahti, E., *Int. J. Cancer*, 13, 579-586 (1974).
- 10 Gahmberg, C. G., Kiehn, D., and Hakomori, S. I., *Nature*, 248, 413-415 (1974).
- 11 Yamada, K. M., and Weston, J. A., *Proc. natn. Acad. Sci. U.S.A.*, 71, 3492-3496 (1974).
- 12 Blumberg, P. M., and Robbins, P. W., *Cell*, 6, 137-147 (1975).
- 13 Estensen, R. D., Hadden, J. W., Hadden, E. M., Touraine, F., Touraine, J. L., Haddox, M. K., and Goldberg, N. D., in *Control of Proliferation in Animal Cells* (edit. by Clarkson, B., and Baserga, R.), 627-634 (Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1974).
- 14 Jacobson, K., Wenner, C. E., Kemp, G., and Papahadjopoulos, D., *Cancer Res.*, 35, 2991-2995 (1975).
- 15 Wigler, M., and Weinstein, I. B., *Nature*, 259, 232-233 (1976).
- 16 Sivak, A., *J. cell. Physiol.*, 80, 167-174 (1972).
- 17 Belman, S., and Troll, W., *Cancer Res.*, 34, 3446-3455 (1974).
- 18 Diamond, L., O'Brien, S., Donaldson, C., and Shimizu, Y., *Int. J. Cancer*, 13, 721-730 (1974).
- 19 Mondal, S., Brankow, D. W., and Heidelberger, C., *Cancer Res.*, 36, 2254-2260 (1976).

Kinetics of lymphocyte stimulation by concanavalin A

AN important problem pertinent to the mechanism of lymphocyte stimulation with a nonspecific mitogen is how long lymphocytes must be exposed to the mitogen to attain maximal stimulation. In the case of the stimulation of human and murine lymphocytes by concanavalin A (con A), the cells are irreversibly committed to stimulation if the lectin is present on the cell membranes for some 18-20 h after the start of the culture¹⁻³. Here we report studies on the kinetics of the stimulation of mouse splenic lymphocytes by con A. Our data indicate that two pulses of con A (0-3 h and 15-18 h after the start of the culture) can stimulate the cells to the same degree as the continuous exposure of the cells to con A for 18 h, suggesting that two signals are necessary for the con A stimulation of lymphocytes. We also show that the first signal is possibly given by the influx of Ca^{2+} .

As shown in Fig. 1, when con A on the membranes of mouse splenic lymphocytes was removed by washing with methyl α -D-mannopyranoside (α -MM) at various times after the start of the culture, the response of the cells as measured by ^3H -thymidine incorporation did not increase linearly with the time of the exposure to con A, but it increased rapidly when the cells were exposed to con A for periods longer

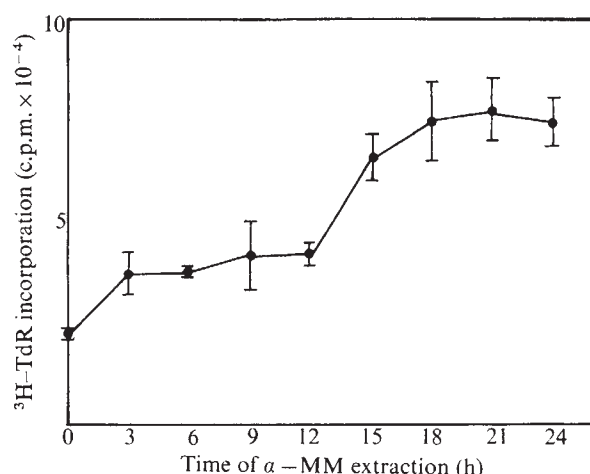


Fig. 1 The effect of con A removal at different times after the start of the culture on the ³H-thymidine incorporation by con A-stimulated mouse splenic lymphocytes. Splenic lymphocytes (2×10^6 cells ml^{-1}) purified from C3H/He mice by Ficoll-Urografin density gradient centrifugation⁵ were incubated in a silicone-coated test tube (1×10 cm) with con A ($4 \mu\text{g ml}^{-1}$) in 1.0 ml of RPMI 1640 medium supplemented with 10% foetal calf serum, 2 mM glutamine and kanamycin ($60 \mu\text{g ml}^{-1}$) at 37°C in a humidified atmosphere of 5% CO_2 and 95% air. At different times, cultures were washed in the culture medium and resuspended in 1.0 ml of 0.05 M α -MM in the culture medium. After 10 min, the cells were collected by centrifugation (300g, 5 min) and resuspended in 1.0 ml of the culture medium. Incorporation of ³H-thymidine ($1.0 \mu\text{Ci}$ per culture; specific activity 23 Ci mmol^{-1}) between 28 and 42 h after the start of the culture was measured by the method described previously⁶. Each point represents the average of three cultures and vertical bars represent ± 1 s.e.m. As control experiments, the incorporation of ³H-thymidine of an unstimulated culture not treated with α -MM ($\square$) and of a culture continuously exposed to con A for 42 h ($\blacksquare$) was shown.

than 12 h. No inhibition was observed when con A was removed at times later than some 18–20 h after the initial binding of the lectin.

We then compared the response after various pulsed exposures to con A with that after the continuous exposure to the lectin for 18 h. Within experimental error, two successive 3-h exposures (0–3 and 15–18 h) separated by an

interval of 12 h yielded the same level of response as continuous exposure for 18 h (Table 1), whereas, when the cells were pulsed with con A only for 3–6 h at the beginning of the culture, or for 3 h between 15 and 18 h, the response was only 25–40% of that seen with an 18-h exposure to con A. These observations were further confirmed by the comparison of the kinetics of DNA synthesis among the cultures which were pulsed with con A at various times. The culture pulsed twice (0–3 and 15–18 h) with con A gave a similar DNA synthesis profile to that of the culture in which the cells were in contact with con A continuously for 18 h (Fig. 2), reaching maximal incorporation of ³H-thymidine by 40 h after the initial binding of con A to the cell membranes. In the other experiments, we shortened the length of the two-pulsed exposure to con A to 1 or 2 h each, but the response was found to be 70–80% of that seen with two 3-h exposures. The requirement of the presence of mitogen on the cell surface at two different stages—at the beginning of culture and at the period just before the onset of DNA synthesis—for maximal lymphocyte stimulation has also been suggested by Weber *et al.*⁴

Since recent investigations have shown that the enhanced uptake of Ca^{2+} is one of the common events which occur within minutes of the binding of mitogen to lymphocytes^{7,8}, we replaced the first exposure to con A (0–3 h) with treatment with calcium ionophore A23187 which has also been shown to be mitogenic to lymphocytes^{9–11}. The data in Table 1 reveal that the pulsed treatment of lymphocytes with A23187 for 3 h at the beginning of the culture followed by a 3-h exposure to con A between 15 and 18 h after the start of the culture is equally effective in the activation of lymphocytes as the two pulses of con A (0–3 and 15–18 h). The addition of EGTA (5×10^{-4} M) to the culture medium with A23187 at the beginning of the culture, however, reduced the stimulation to 60–70%. Furthermore, the response of the culture pulsed with con A first (0–3 h) and then with A23187 (15–18 h) was much smaller than that of the culture pulsed with A23187 first and with con A later.

These results suggest that the Ca^{2+} influx into the cell mediated by ionophore or induced by the mitogenic lectin on the cell membranes possibly plays an important part in the triggering of lymphocyte stimulation as the first signal. In view of the recent findings that guanylate cyclase activity of lymphocytes is dependent on the intracellular

Table 1 Effect of pulsed exposure of lymphocytes to con A and calcium ionophore A23187

Time (h)						³ H-Thymidine incorporation (c.p.m.)
0	3	9	12	15	18	
—C—				—C—		11,219 ± 391
—C—		—C—		—C—		17,090 ± 568
—C—				—C—		21,360 ± 3,235
—C—		—C—		—C—		37,038 ± 3,947
—A—					—C—	34,035 ± 88
—A—					—A—	11,492 ± 178
—A—				—A—		14,755 ± 866
—A—				—A—		15,318 ± 1,211
—A—		—A—		—A—		16,452 ± 1,354
—C—				—A—		21,852 ± 1,393
—A—				—C—		36,465 ± 4,390
—A—				—C—		29,259 ± 2,499
Cell control						9,398 ± 1,975

Mouse splenic lymphocytes were cultured with con A ($4 \mu\text{g ml}^{-1}$) or with A23187 ($0.05 \mu\text{g ml}^{-1}$) as described in the legend of Fig. 1. For the removal of con A and A23187, the following washing procedure was carried out in both cases. At an appropriate time after the start of the culture, cells were washed once in the culture medium and resuspended in 1.0 ml of 0.05 M α -MM in the culture medium. After 10 min, the cells were collected by centrifugation (300g, 5 min) and resuspended in 1.0 ml of the culture medium. Incorporation of ³H-thymidine between 39 and 42 h after the start of the culture was measured by the method previously described⁶. Each value represents the mean $\pm$ s.e.m. of three cultures. The horizontal bars represent the time during which con A (C) or A23187 (A) was present.

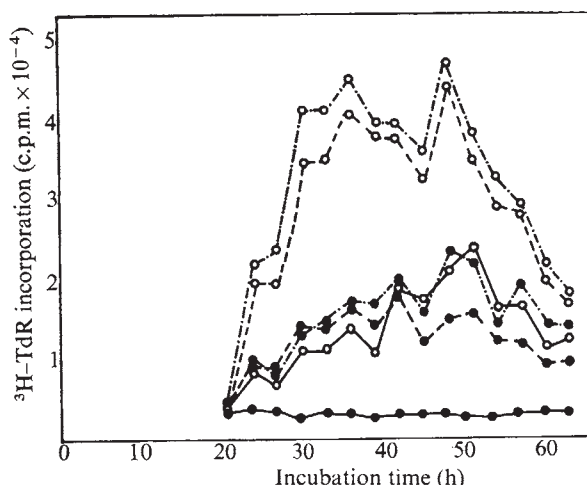


Fig. 2 The effect of length of exposure to con A on the kinetics of DNA synthesis in mouse splenic lymphocyte cultures. Culture of lymphocytes and the removal of con A from each culture were performed as described in the legend of Fig. 1. ○, Control cultures without con A. Periods for which the cultures were incubated with con A ($4 \mu\text{g ml}^{-1}$). □, 0–3; ■, 0–6; ●, 15–18; ▲, 0–3+15–18; △, 0–18 h. DNA synthesis was measured with a terminal 3-h pulse of ^3H -thymidine ($2.5 \mu\text{Ci}$ per culture). Each point represents the average of three cultures.

level of calcium ion¹² and microtubule function is controlled by the local concentration of Ca^{2+} in the cell cytoplasm^{13,14}, the mitogen-induced influx of Ca^{2+} into lymphocytes triggers the stimulation of the cells possibly by the elevation of cyclic GMP level in the cells and/or the functional alteration of microtubular assembly which modulates the topography of cell-surface receptors. After approximately 12–15 h from the influx of the first signal, the cells attain a preactivated state, and these then proceed to the DNA synthetic period (S phase) if the second signal is provided by the presence of mitogen on the cell membrane. It may, however, be necessary to estimate actual numbers of responding cells during the course of the exposure to con A to validate this assumption further. Investigation of the nature of the second signal and its triggering mechanism of lymphocyte activation.

This work was supported by a grant from the Ministry of Education, Science and Culture of Japan.

SATOSHI TOYOSHIMA
MAKOTO IWATA
TOSHIKI OSAWA

Division of Chemical Toxicology and Immunochemistry,
Faculty of Pharmaceutical Sciences,
University of Tokyo,
Bunkyo-ku, Tokyo 113, Japan

Received May 24, accepted October 4, 1976.

- ¹ Lindahl-Kiessling, K., *Expl Cell Res.*, **70**, 17–26 (1972).
- ² Novogrodsky, A., and Katchalski, E., *Biochim. biophys. Acta*, **228**, 579–583 (1971).
- ³ Gunter, G. R., Wang, J. L., and Edelman, G. M., *J. Cell Biol.*, **62**, 366–377 (1974).
- ⁴ Weber, Th. H., Skoog, V. T., Mattsson, A., and Lindahl-Kiessling, K., *Expl Cell Res.*, **85**, 351–361 (1974).
- ⁵ Kawaguchi, T., Matsumoto, I., and Osawa, T., *J. Biol. Chem.*, **249**, 2786–2792 (1974).
- ⁶ Toyoshima, S., Akiyama, Y., Nakano, K., Tonomura, A., and Osawa, T., *Biochemistry*, **10**, 4457–4463 (1971).
- ⁷ Parker, C. W., *Biochem. biophys. Res. Commun.*, **61**, 1180–1186 (1974).
- ⁸ Freedman, M. H., Raff, M. C., and Gomperts, B., *Nature*, **255**, 378–382 (1975).
- ⁹ Maino, V. C., Green, N. M., and Crumpton, M. J., *Nature*, **251**, 324–327 (1974).
- ¹⁰ Luckasen, J. R., White, J. G., and Kersey, J. H., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 5088–5090 (1974).
- ¹¹ Hovi, T., Allison, A. C., and Williams, S. C., *Expl Cell Res.*, **96**, 92–100 (1976).
- ¹² Katagiri, T., Terao, T., and Osawa, T., *J. Biochem. (Tokyo)*, **79**, 849–852 (1976).
- ¹³ Gillespie, G., *J. Cell Biol.*, **50**, 544–549 (1971).
- ¹⁴ Gallin, J. I., and Rosenthal, A. S., *J. Cell Biol.*, **62**, 594–609 (1974).

Rat platelets aggregate in the absence of endogenous precursors of prostaglandin endoperoxides

THE prostaglandin (PG) endoperoxides, PGG_2 and PGH_2 , are thought to have a function in human platelet aggregation, since they are formed during thrombin-induced aggregation¹. They induce aggregation on addition to platelet-rich plasma (PRP), either directly or after conversion to thromboxane A_2 (TXA_2)². TXA_2 (half life ~ 30 s) is formed *in vitro* by a microsomal enzyme which is insensitive to inhibition by aspirin-like drugs^{3,4}. It is therefore distinct from the cyclo-oxygenase that converts arachidonic acid (AA) into PG endoperoxides. AA, after incorporation into membrane phospholipids, is liberated during thrombin-induced aggregation and is then almost completely metabolised⁵ by the cyclo-oxygenase and AA-lipoxygenase⁶. Therefore, we tested platelets of animals that were deprived of AA, and still observed aggregation with collagen, indicating that PG endoperoxides and TXA_2 are not essential for aggregation.

Feeding mammals with an essential fatty acid-deficient (EFAD) diet, lacking linoleic acid ($18:2$, $n-6$), results in a decrease of *bis*-homo- γ -linolenic acid ($20:3$, $n-6$) and AA ($20:4$, $n-6$), both PG precursors. The part played by AA in membrane phospholipids is replaced by eicosatrienoic acid ($20:3$, $n-9$), which is not a substrate⁷ but a competitive inhibitor⁸ of the cyclo-oxygenase. Pregnant rats (Wistar, TNO, Zeist, The Netherlands) were placed on a diet containing 4% hydrogenated cocofat (Hope Farms), 5 d before the expected day of delivery⁹. After weaning, the newborn rats were kept on EFAD food; controls received 3.5% of their calories as linoleic acid. Apart from reduced growth¹⁰, the EFAD condition of the rats was clearly demonstrated by fatty acid analysis of erythrocytes (Table 1). A marked reduction of the linoleic acid family ($18:2$, $n-6$; $20:3$, $n-6$; $20:4$, $n-6$) was observed in favour of the oleic acid ($18:1$, $n-9$) family. The criterion of Holman¹⁰ for EFA deficiency—a ratio between eicosatrienoic acid ($20:3$, $n-9$) and AA ($20:4$, $n-6$) greater than 0.4—was amply exceeded.

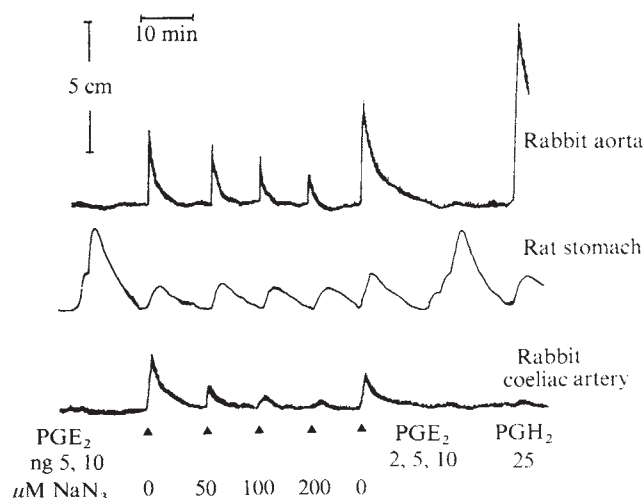


Fig. 1 Differences in bioassay of rat platelet RCS and PGH_2 . The cascade, with rabbit aorta, rat stomach and rabbit coeliac artery strips, was superfused with a Krebs' solution (2.5 ml min^{-1} , 37°C) containing a mixture²¹ of antagonists to inactivate serotonin, histamine, acetylcholine and catecholamines and indomethacin ($3 \mu\text{M}$) to prevent PG synthesis by the tissues. Cumulative²¹ doses of PGE_2 , and PGH_2 in glucose-free Krebs', were superfused over the tissues. Samples (0.1 ml) of normal PRP, preincubated for 3 min at 37°C with different concentrations of NaN_3 , were superfused directly (▲) on the cascade, 1 min after addition of collagen ($40 \mu\text{g ml}^{-1}$). Preparation of PRP and aggregation was performed as described in Table 2.

Table 1 Fatty acid composition of total fat from normal and EFAD rat erythrocytes

Fatty acid	Normal % weight	EFAD % weight
16:0	29.8 ± 1.3	28.9 ± 0.9
18:0	20.0 ± 1.5	17.5 ± 2.1
18:1, <i>n</i> -9	10.2 ± 1.1	19.8 ± 0.7
18:2, <i>n</i> -6	7.6 ± 0.7	0.8 ± 0.04
20:3, <i>n</i> -6	0.2 ± 0.04	< 0.1
20:3, <i>n</i> -9	< 0.1	8.7 ± 0.9
20:4, <i>n</i> -6	18.6 ± 1.7	4.5 ± 0.6
<i>R</i> = 20:3, <i>n</i> -9 20:4, <i>n</i> -6	< 0.013 ± 0.002	1.97 ± 0.09

Heparinised blood was obtained by cardiac puncture from male rats (23–25 weeks old) under ether anaesthesia. After centrifugation (30 min, 950g) plasma and 'buffy coat' were removed and the erythrocytes were washed three times with 6 volumes of 10 mM EDTA in saline. Total fat of 5 ml packed cells was isolated²⁴ by two extractions with 19 volumes of chloroform-methanol (2:1). After saponification of 5 mg lipid, the fatty acids were extracted (pH=4) with pentane (3 × 25 ml), dried over anhydrous Na₂SO₄ and methyl esters were prepared in a fresh ethereal diazomethane solution (nitrogen atmosphere). The fatty acid pattern was determined with a Hewlett Packard 5750 gas chromatograph, equipped with a 4 foot × 3-mm column (3% EGSS-X on gaschrom Q; Chrompack, The Netherlands) and a flame-ionisation detector. The equivalent chain length values were compared with those of a mixture of standard fatty acids and with the data of Hofstetter²⁵. Weight percentages were determined by triangulation, and expressed as means (± s.e.) of four rats.

Aggregation induced by different stimuli was investigated in both normal and EFAD PRP (Table 2) and no significant differences in platelet number were observed *in vivo*¹¹ or in PRP. In rat platelets adenosine diphosphate (ADP) induces only primary aggregation¹², which is probably independent of the PG-endoperoxide mechanisms¹³. Therefore, a supra-maximal dose of ADP (40 μM) was added after each aggregation, serving as control for the aggregatory capacity of each sample. ADP induced equal aggregation in normal and EFAD PRP, except at the lowest dose, when EFAD PRP aggregated less (56%) than normal PRP. Although electron microscopy did not reveal membrane differences, this result might reflect an altered membrane structure¹⁴.

The sensitivity of rat PRP towards the PG-endoperoxide PGH₂ seemed to be low. At low doses (from 500 ng ml⁻¹) PGH₂ only induced a minor shape change as seen from a small decrease in light transmission and diminished oscillations. With 1 μg ml⁻¹ a small transient aggregation was observed. Only small amounts of PGH₂ may enter the rat cells and therefore may not be converted to TXA₂ by the internal microsomal enzyme system. Preloading of PRP with a high concentration of linoleic acid, in order to eliminate binding of PGH₂ to albumin¹⁵, did not alter rat PRP responses. No significant differences were observed between EFAD and normal PRP (*P* > 0.05, analysis of variance) in any condition.

EFA deficiency did not alter the aggregating efficacy of high collagen doses, but inhibited almost completely aggregation by a dose giving half maximal responses in normal PRP. Since feedback inhibition by PGD₂, the most potent inhibitor of human platelet aggregation^{16,17}, is absent in rat platelets¹⁷, the diminished response from platelets with a low internal AA content might be caused by a rate-limiting production of PG-endoperoxides and TXA₂. To check this assumption the release of substances derived from AA and involved in aggregation was assessed directly on a cascade¹⁸ of isolated tissues¹⁹.

The substances formed by aggregating normal rat PRP, were differentiated by using a combination of rabbit aorta^{19,21}, rat stomach^{18,19} and rabbit coeliac artery²⁰ strips, in the presence of appropriate antagonists²¹ (Fig. 1). PRP, 1 min after addition of collagen (40 μg ml⁻¹) contracted all tissues. All smooth muscle-stimulating activities, including rabbit aorta contracting substance¹⁹ (RCS) were lipid soluble, because they were removed after passage through a small Amberlite XAD-2

column, as shown previously²¹. Rabbit coeliac artery has been reported to contract to TXA₂ and to show relaxation towards PGE₂ and PGH₂ (ref. 20). The latter observation was not confirmed and small contractions were observed with higher doses of PGH₂. TXA₂ may be generated by aggregating PRP since rabbit coeliac artery shows great sensitivity towards the contractile activity of TXA₂ (ref. 20).

Rabbit aorta is sensitive towards PGH₂, but TXA₂ is far more potent than the PG-endoperoxides^{3,4,20}. Although comparison of sets of rabbit aorta and coeliac artery responses towards control PRP (0 μM NaN₃) indicates that a TXA₂-PG-endoperoxide balance seems to exist, the main component of RCS is probably TXA₂. This was confirmed by the half life of RCS at 37 °C, 32 ± 6 s (*n* = 5), following removal of platelets by quick filtration through a Millipore filter to avoid new TXA₂ formation. The half life of PGH₂, stirred in platelet-poor plasma (900 r.p.m., 37 °C), was 125 ± 42 s (*n* = 8). These half lives were estimated from linear plots of the logarithms of rabbit aorta contractions at different times. Finally, inhibition of RCS by low doses of NaN₃, without altering the rat stomach contractions, again indicated that RCS differs from PG-endoperoxides. Rat stomach is stimulated by PGE₂, to a lesser extent by PG-endoperoxides²¹ and PGD₂, but hardly at all by TXA₂ (refs 3, 4, 20). The mechanism of azide interference with TXA₂, also demonstrated²² in rabbit PRP aggregated by AA, remains obscure. Possible explanations are inactivation of the TXA₂-forming microsomal system or an enhanced conversion of TXA₂ to the N₃ analogue of the inactive TXB₂ (ref. 2). As expected, no differences were observed in IC₅₀ values for TXA₂ and PGE inhibition by the cyclo-oxygenase inhibitors indomethacin and eicosatetraynoic acid, because both act at a stage before TXA₂ formation (unpublished results).

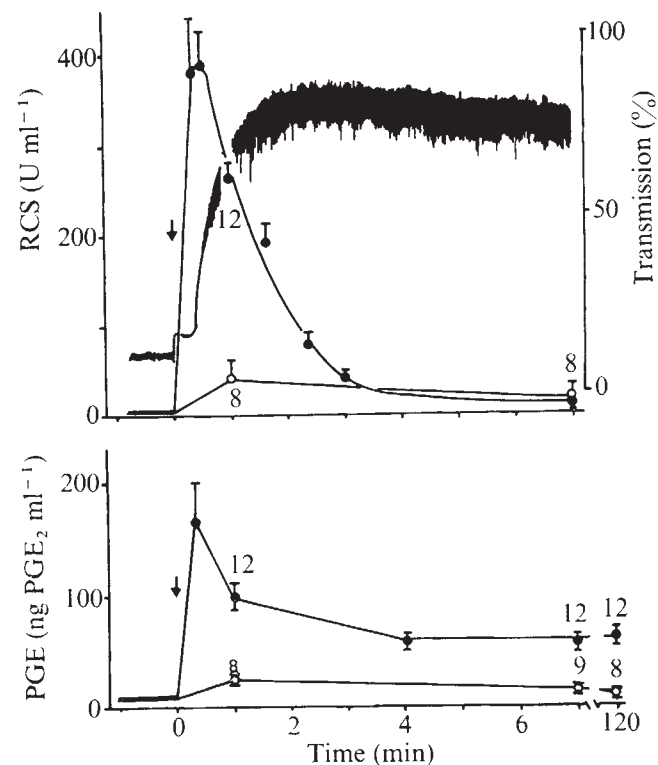


Fig. 2 Time courses of aggregation and generation of RCS (mainly TXA₂), PG endoperoxides and PGE by aggregating normal (●) and EFAD (○) PRP. Samples (0.1 ml) of rat PRP, aggregating after addition of 40 μg ml⁻¹ collagen (arrow) were superfused directly on rabbit aorta, and rat stomach strips (see legend Fig. 1). The upper part shows the changes in light transmission during aggregation of normal PRP together with release of RCS (expressed in units, 1 unit = 1 ng PGH₂ ml⁻¹). The lower part shows rat stomach contractions, calibrated with PGE₂. Each value is the mean (± s.e.) of four experiments, unless indicated otherwise.

Table 2 Comparison between the aggregatory capacity of normal and EFAD platelet-rich plasma (PRP)

	Normal PRP	EFAD PRP
Platelet count 10 ³ cells µl ⁻¹	618 ± 38 (5)	729 ± 31 (5)
Aggregation inducer	% Aggregation	% Aggregation
ADP 2.50 µM	95.7 ± 2.6 (5)	90.8 ± 1.5 (5)
0.80 µM	76.9 ± 5.4 (5)	75.7 ± 3.9 (5)
0.25 µM	25.9 ± 3.0 (5)	14.5 ± 2.4 (5)*
PGH ₂ 1 µg ml ⁻¹	25.0 ± 5.5 (4)	17.9 ± 4.0 (3)
Linoleic acid (10 mM) + PGH ₂ 1 µg ml ⁻¹	14.7 ± 4.3 (3)	15.5 ± 2.7 (3)
Collagen 40 µg ml ⁻¹	95.2 ± 1.6 (5)	95.3 ± 1.6 (5)
4 µg ml ⁻¹	53.6 ± 10.3 (5)	3.1 ± 1.5 (5)†

PRP was collected after centrifugation (15 min, 200g, room temperature) of heparinised blood (see Table 1) and diluted with platelet-poor plasma (30 min, 950g) to A 602 nm/1 cm = 0.9. PRP was prepared daily, avoiding any glass contact and stored at 0 °C. The platelet number was determined with a Coulter counter (Model A). PRP (200 µl) was preincubated (3 min, 900 r.p.m., 37 °C) with 25 µl Tyrode (glucose and Ca²⁺-free) or with 25 µl Tyrode containing linoleic acid. Aggregation was started by addition of 25 µl inducer and maximal increase in light transmission was measured (Payton Single Channel Module, Payton Ass. Inc., Buffalo, New York). Results are expressed as percentages of maximal increase with 40 µM ADP, added in 10 µl 5 min after starting aggregation. Collagen (Bovine achilles tendon, Sigma, final concentration 400 µg ml⁻¹) was suspended in 18 mM acetic acid. PGH₂ was stored at -70 °C in diethylether and after evaporation, it was immediately dissolved in Tyrode and tested. ADP (Boehringer) and linoleic acid (Merck) were also dissolved in Tyrode. The values are the means (± s.e.) of the indicated number of experiments.

* $P < 0.05$, † $P < 0.01$, Wilcoxon test, two-sided.

Release of TXA₂, PG-endoperoxides and PGE by normal and EFAD PRP, together with an aggregation pattern of normal PRP, is shown in Fig. 2. Although RCS is mainly TXA₂, the rabbit aorta strips were calibrated with PGH₂. No activity was detectable during preincubation. At 20 s after collagen addition, when aggregation is starting after a lag period, TXA₂ was almost maximally generated by normal PRP. TXA₂ concentration was practically unaltered at 30 s, when light transmission increased steeply. At 1 min, however, when aggregation is completed for 75%, TXA₂ is already disappearing and when maximal increase in light transmission, together with oscillations due to aggregates, was observed (3 min), hardly any TXA₂ was detectable. EFAD PRP generated only 16% of the TXA₂ released by normal PRP 1 min after collagen, but aggregation of EFAD PRP was unaltered with this high collagen dose (also shown in Table 2). PGE formation showed the same pattern, with the exception that a residual activity was observed which was stable for up to 2 h after aggregation. This activity behaved like PGE after extraction, column chromatography and differential bioassay on rat stomach strip and rat colon (H. B. and I. L. B., in preparation). Since TXA₂ is not spasmogenic on rat stomach³, the initial peak of activity was probably composed mainly of PG-endoperoxides. These compounds degrade to PGE₂ (ref. 23) and other catabolites. Again EFAD PRP generated less PGE (20% or less when assessed at 7 min or later) and less unstable PG-endoperoxide-like activity (24%, 1 min) than normal PRP. The decreased formation of TXA₂ and PGE by EFAD platelets was not a result of decreased cyclo-oxygenase activity. During AA (0.325 mM) induced aggregation PG-endoperoxide-like activity (1 min) and PGE (7 min) were formed in equal amounts by normal and EFAD PRP.

Although this differential bioassay is less accurate than gas-liquid chromatography-mass spectrometry of degradation products of TXA₂ and PG-endoperoxides, it gives a dynamic picture of the presence of separate biologically active substances during platelet aggregation. These data, obtained with platelets in a more physiological environment than in other studies^{2,5}, indicate that the capacity of platelets to aggregate with threshold

doses of collagen is dependent on the availability of endogenous AA. Decreased generation of TXA₂ and PG-endoperoxides coincides with diminished aggregation by EFAD platelets, although their sensitivity towards PG-endoperoxides and their cyclo-oxygenase activity are unchanged. With high collagen concentrations the PG-endoperoxide generating system is replaced by other mechanisms because a reduction of endogenous AA did not prevent platelet aggregation. Finally, specific inhibition of TXA₂ formation, as shown with sodium azide, might provide opportunities to reduce platelet aggregation without affecting the formation of PGD₂, which has strong anti-thrombotic effects in human platelets.

We thank Drs J. E. Vincent for discussions, A. Montfoort for assistance in fatty acid analysis, J. J. Michiels for preparation of electron micrographs, D. H. Nugteren (Unilever Research, Vlaardingen, The Netherlands) for gifts of PGH₂, and J. E. Pike (Upjohn company, Kalamazoo) for gifts of PGE₁, PGE₂ and PGF_{2α}. Mr F. J. Zijlstra and Miss A. L. Heisterkamp gave technical assistance and Dr M. Parnham read the manuscript. H. B. was supported by the Dutch Foundation for Medical Research, FUNGO.

H. BULT

I. L. BONTA

Department of Pharmacology,
Erasmus University,
Rotterdam, The Netherlands

Received August 24; accepted September 28, 1976.

- 1 Hamberg, M., Svensson, J., Wakabayashi, T., and Samuelsson, B., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 345-349 (1974).
- 2 Hamberg, M., Svensson, J., and Samuelsson, B., *Proc. natn. Acad. Sci. U.S.A.*, **72**, 2994-2998 (1975).
- 3 Needleman, P., *et al.*, *Nature*, **261**, 558-560 (1976).
- 4 Needleman, P., Minkes, M., and Raz, A., *Science*, **193**, 163-165 (1976).
- 5 Bills, T. K., Smith, J. B., and Silver, M. J., *Biochim. biophys. Acta.*, **424**, 303-315 (1976).
- 6 Nugteren, D. H., *Biochim. biophys. Acta.*, **380**, 299-307 (1975).
- 7 Dorp, D. A. van, *Ann. N.Y. Acad. Sci.*, **180**, 181-199 (1971).
- 8 Ziboh, V. A., Vanderhoek, J. Y., and Lands, W. E. M., *Prostaglandins*, **5**, 233-240 (1974).
- 9 Bonta, I. L., Chrispijn, H., Noordhoek, J., and Vincent, J. E., *Prostaglandins*, **5**, 495-503 (1974).
- 10 Holman, R. T., *J. Nutr.*, **76**, 405-410 (1960).
- 11 Vincent, J. E., Zijlstra, F. J., and Bonta, I. L., *Prostaglandins*, **10**, 899-911 (1975).
- 12 MacIntyre, D. E., and Gordon, J. L., *Biochem. Soc. Trans.*, **2**, 1265-1269 (1974).
- 13 Zucker, M. B., and Peterson, J., *Proc. Soc. exp. Biol. Med.*, **127**, 547-551 (1968).
- 14 Farias, R. N., Bloy, B., Morero, R. D., Sinerez, F., and Tucco, R. E., *Biochim. biophys. Acta.*, **415**, 231-251 (1975).
- 15 Hamberg, M., and Fredholm, B. B., *Biochim. biophys. Acta.*, **431**, 189-193 (1976).
- 16 Nishizawa, E. E., *et al.*, *Prostaglandins*, **9**, 109-121 (1975).
- 17 MacIntyre, D. E., and Gordon, J. L., *Nature*, **258**, 337-339 (1975).
- 18 Vane, J. R., *Br. J. Pharmac.*, **23**, 360-373 (1964).
- 19 Piper, P. J., and Vane, J. R., *Nature*, **223**, 29-35 (1969).
- 20 Bunting, S., Moncada, S., and Vane, J. R., *Br. J. Pharmac.*, **57**, 462P-463P (1976).
- 21 Bult, H., and Bonta, I. L., *Agents and Actions*, **6** (in the press).
- 22 Vargaftig, B. B., Tranier, Y., and Chignard, M., *Eur. J. Pharmac.*, **33**, 19-29 (1975).
- 23 Willis, A. L., *Prostaglandins*, **5**, 1-25 (1974).
- 24 Folch, J., Lees, M., and Sloane-Stanley, G. H., *J. biol. Chem.*, **226**, 497-509 (1957).
- 25 Hofstetter, H. H., Sen, N., and Holman, R. T., *J. Am. Oil chem. Soc.*, **42**, 537-540 (1965).

Stimulation of 'irritant' receptors and afferent C-fibres in the lungs by prostaglandins

THE lungs are among the many organs that generate, release and destroy prostaglandins (PGs)¹. It has been suggested that PGs act as 'local hormones'², so that prostaglandins E₁ and E₂, which relax the smooth muscle of bronchi and pulmonary blood vessels in many species, and F_{2α}, which contracts it, may be implicated in normal regulation of airway and pulmonary vascular calibre³. PGF_{2α} has been proposed as a causal factor in asthma⁴. The bronchoconstrictor effect of PGF_{2α} is reduced by atropine, hence a reflex component may be involved⁵. Cough and airway irritation have been reported in clinical trials of PGE₁ and PGE₂ as bronchodilators in the treatment of asthma^{6,7}. Thus PGs may stimulate afferent nerve endings in the lungs, and there has been speculation as to which endings are involved. We have recorded impulse activity from rapidly-adapting pulmonary stretch ('irritant') receptors^{8,9} and afferent C-fibre endings^{10,11} in the lungs of anaesthetised

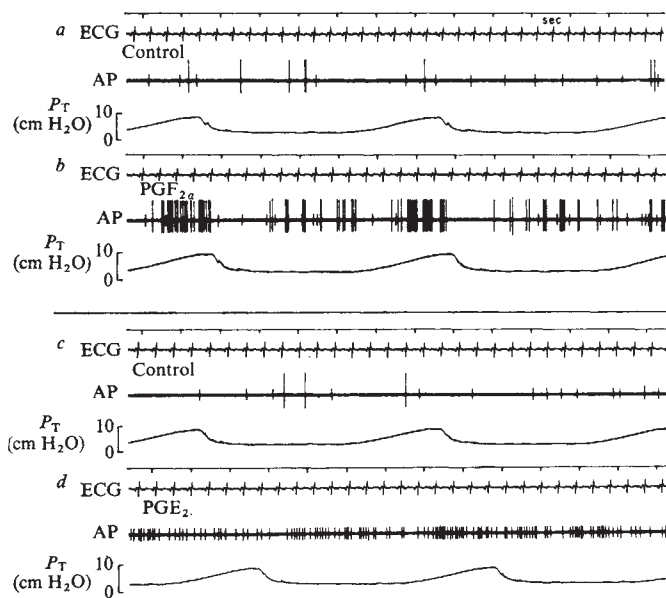


Fig. 1 Comparison of the effects of $\text{PGF}_{2\alpha}$ and PGE_2 on an 'irritant' receptor (large spikes) and a C-fibre ending (small spikes). Both endings were located in the lower lobe of the left lung. Conduction velocity was 27.2 m s^{-1} in the 'irritant' fibre, and 1.3 m s^{-1} in the C-fibre. *a*, Before, and *b*, 16 s after injection of $\text{PGF}_{2\alpha}$ ($4 \mu\text{g kg}^{-1}$) into the right atrium. Interval of 6 min between *b* and *c*. *c*, Before, and *d*, 42 s after injection of PGE_2 ($20 \mu\text{g kg}^{-1}$) into the right atrium. From above downwards in each record: 1 s time trace; ECG, electrocardiogram; AP, action potentials recorded from an afferent filament of the left vagus nerve; P_T , tracheal pressure (upstroke representing inflation).

dogs. When $\text{PGF}_{2\alpha}$ was injected into the right atrium, lung 'irritant' receptors were strongly stimulated. Injection of PGEs, by contrast, caused marked and prolonged stimulation of lung C-fibres.

We recorded impulses from fine strands (containing one, or at most two, active fibres) of the cervical vagus nerve in dogs anaesthetised with Dial-Nembutal. The two types of lung afferent were identified by their patterns of discharge, by their responses to various procedures (for example hyperinflation of the lungs; injection of chemicals known to stimulate them), and by the conduction velocity of their fibres⁸⁻¹¹. The chest was open so that we could determine by direct mechanical stimulation the location of the ending whose impulses we had recorded. The lungs were ventilated at constant tidal volume by a positive pressure pump. Tidal CO_2 was monitored continuously, and maintained within normal limits. We measured systemic and pulmonary arterial blood pressure and tracheal pressure. Drugs were injected into the right and left atrium, or administered as aerosols generated by an ultrasonic nebuliser (DeViblis). Some afferent vagal C-fibre endings in the lung are located in structures supplied by the pulmonary circulation, others are in structures supplied by the bronchial circulation¹¹. However, PGs had similar effects on these two groups of C-fibres, and the results have been combined.

Injection of $\text{PGF}_{2\alpha}$ ($1-8 \mu\text{g kg}^{-1}$) into the right atrium stimulated all 23 'irritant' receptors examined. These doses are within the range used by Wasserman⁶ to produce changes in airway mechanics in anaesthetised dogs. In the majority of experiments reported here we did not examine the dose-response relationship in detail because the recording life of the fine vagal strands was often limited, and we had to determine the precise location of the ending before the fibre became inactive. In most cases, therefore, we used a dose of $4 \mu\text{g kg}^{-1}$ to examine the response of the ending. Latencies of onset ranged from 5 to 25 s (mean 12.2). Activity increased from

$1.7 \pm 0.4 \text{ impulses s}^{-1}$ (mean $\pm$ s.e.) during the control period to $10.4 \pm 2.0 \text{ impulses s}^{-1}$, when averaged over one respiratory cycle at the peak of the response. Firing remained above control for 30-120 s (mean 73.5). There was invariably an increase in peak tracheal pressure ($2.1 \pm 0.3 \text{ cm H}_2\text{O}$). The observed cardiovascular effects, consisting of an increase in pulmonary arterial pressure, and usually a small rise in systemic arterial pressure, confirm earlier findings¹². 'Irritant' receptor firing and tracheal pressure also increased when an aqueous solution of $\text{PGF}_{2\alpha}$ ($0.05-0.1 \text{ mg ml}^{-1}$) was administered as an aerosol. A typical response to $\text{PGF}_{2\alpha}$ injected into the right atrium is seen in Fig. 1.

The majority of 'irritant' receptors are believed to be in the larger airways¹³, which are supplied by the bronchial circulation. Nevertheless, injection of $\text{PGF}_{2\alpha}$ ($2-12 \mu\text{g kg}^{-1}$) into the left atrium stimulated only one out of eight receptors tested; tracheal pressure increased on average by only $0.2 \text{ cm H}_2\text{O}$, and arterial pressures were unchanged. Thus $\text{PGF}_{2\alpha}$ stimulates 'irritant' receptors regularly only when it has access to the pulmonary circulation, from which site it produces its maximal bronchoconstrictor effects. This observation, coupled with evidence that PGs are largely destroyed in a single passage through the lung¹, favours the hypothesis that 'irritant' receptor stimulation is not primarily due to the effects of $\text{PGF}_{2\alpha}$ on the nerve ending, but is secondary to changes in lung mechanics. This interpretation was strengthened by our finding that administration of an aerosol of isoproterenol ($0.1-0.2 \text{ mg ml}^{-1}$) significantly reduced the effect of $\text{PGF}_{2\alpha}$ on both receptor activity and tracheal pressure (Table 1). Similarly, when PGE_2 ($10-20 \mu\text{g kg}^{-1}$), which has been shown to reduce the bronchoconstrictor effect of $\text{PGF}_{2\alpha}$ ^{14,15}, was injected into the right atrium 1-6 min before injection of $\text{PGF}_{2\alpha}$, there was a significant reduction in the effect of $\text{PGF}_{2\alpha}$ on both receptor firing and tracheal pressure. However, in experiments in which we used isoproterenol, PGE_2 , or atropine to antagonise the bronchoconstrictor effects of $\text{PGF}_{2\alpha}$, there were a few instances in which 'irritant' receptor stimulation by $\text{PGF}_{2\alpha}$ was unchanged. Hence the possibility that $\text{PGF}_{2\alpha}$ also stimulates 'irritant' receptors directly cannot be ruled out.

If $\text{PGF}_{2\alpha}$ has a causative role in asthma⁴, then our finding that $\text{PGF}_{2\alpha}$ stimulates 'irritant' receptors acquires considerable pathophysiological significance. It has been postulated that stimulation of 'irritant' receptors by histamine causes a reflex bronchoconstriction which is superimposed on the direct action of the drug^{16,17}. The same may be true for $\text{PGF}_{2\alpha}$.

There was a similar but less pronounced stimulation of 14 of 19 lung C-fibres when $\text{PGF}_{2\alpha}$ ($2-12 \mu\text{g kg}^{-1}$) was injected into the right atrium, impulse frequency for the group as a whole

Table 1 Reduction of effects of $\text{PGF}_{2\alpha}$ by isoproterenol aerosol

		'Irritant' receptor activity (impulses s^{-1})	Peak tracheal pressure (cm H_2O)
A	Control	1.4 ± 0.5	10.9 ± 0.5
	$\text{PGF}_{2\alpha}$	12.6 ± 2.1	12.9 ± 0.7
B	Control	2.1 ± 0.6	10.9 ± 0.5
	$\text{PGF}_{2\alpha}$	4.5 ± 0.7	11.2 ± 0.5

Effects on 'irritant' receptor activity and tracheal pressure of injecting $\text{PGF}_{2\alpha}$ ($2-8 \mu\text{g kg}^{-1}$) into the right atrium. Results comprise 13 complete trials on eight receptors. The data in the upper two lines (labelled A) were obtained before administration of isoproterenol; data in the lower two lines (labelled B) were obtained after an aerosol of isoproterenol ($0.1-0.2 \text{ mg ml}^{-1}$ solution) had been administered for 5 min. Mean values are given together with their standard errors. The reduction by isoproterenol of the effects of $\text{PGF}_{2\alpha}$ was highly significant ($P < 0.002$).

increasing from 0.5 ± 0.1 to 2.1 ± 0.5 impulses s^{-1} (Fig. 1). The latency of response was 24 ± 2.5 s, and firing remained above control levels for 123 ± 20.8 s.

Surprisingly, PGs of the E series, which are known to be highly irritant^{6,7}, had only minor effects on 'irritant' receptors, only 6 of 12 receptors being stimulated by PGE₂ ($10\text{--}20 \mu\text{g kg}^{-1}$) injected into the right atrium. The increase in activity for the group as a whole was only 1.4 ± 0.5 impulses s^{-1} and the effect lasted no more than four to five respiratory cycles. On average, peak tracheal pressure decreased by $0.1 \text{ cm H}_2\text{O}$. PGE₂ ($5\text{--}10 \mu\text{g kg}^{-1}$ injected into the right atrium), however, was a powerful stimulant of 19 of 21 lung C-fibre endings, impulse activity increasing from 0.6 ± 0.1 to 5.9 ± 0.9 impulses s^{-1} (with a latency of response of 14.7 ± 0.8 s) (Fig. 1). The effect, moreover, was extremely prolonged (average duration 6.5 min); indeed 15 min sometimes elapsed before firing returned to control levels. This was surprising in view of the reported rapid inactivation of PGs¹. The concomitant hypotensive effect also was long-lasting, a finding that agrees with earlier observations on PGE₁¹². We have no explanation for these prolonged effects.

In the same dose range, PGE₁ had similar but less pronounced effects on C-fibre endings. The relative potencies of PGE₁ and PGE₂ on C-fibre endings parallel the observation that, in man, the irritant properties of PGE₁ are less than those of PGE₂ (ref. 7). Furthermore, in our experiments, administration of isoproterenol aerosol did not reduce the stimulation of lung C-fibre endings by PGE₂, and in man isoproterenol does not prevent the cough response to PGE₂ (ref. 6). Hence it is conceivable that C-fibres, rather than 'irritant' receptors, are responsible for PGE-induced cough.

We thank Dr John Pike of the Upjohn Company for the prostaglandins used in these experiments. This work was supported by a grant from the National Heart and Lung Institute.

H. M. COLERIDGE
J. C. G. COLERIDGE
K. H. GINZEL
D. G. BAKER
R. B. BANZETT
M. A. MORRISON

Cardiovascular Research Institute,
University of California San Francisco,
San Francisco, California 94143

Received June 28; accepted October 4, 1976.

- 1 Piper, P., and Vane, J., *Ann. N. Y. Acad. Sci.*, **180**, 363–383 (1971).
- 2 Weeks, J. R., *Fedn Proc.*, **33**, 37–47 (1974).
- 3 Fanburg, B. L., *Am. Rev. Resp. Dis.*, **108**, 482–489 (1973).
- 4 Parker, C. W., and Snider, D. E., *Ann. intern. Med.*, **78**, 963–965 (1973).
- 5 Wasserman, M. A., *Eur. J. Pharmac.*, **32**, 146–155 (1975).
- 6 Herxheimer, H., and Roetscher, I., *Eur. J. clin. Pharmac.*, **3**, 123–125 (1971).
- 7 Kawakami, Y., Uchiyama, K., Irie, T., and Murao, M., *Eur. J. clin. Pharmac.*, **6**, 127–132 (1973).
- 8 Sampson, S. R., and Vidruk, E. H., *Respir. Physiol.*, **25**, 9–22 (1975).
- 9 Mills, J. E., Sellick, H., and Widdicombe, J. G., *J. Physiol., Lond.*, **203**, 337–357 (1969).
- 10 Coleridge, H. M., Coleridge, J. C. G., and Luck, J. C., *J. Physiol., Lond.*, **179**, 248–262 (1965).
- 11 Coleridge, H. M., and Coleridge, J. C. G., in *Krogh Centenary Symposium on Capillary Exchange, Pulmonary Oedema and Respiratory Adaptations* (edit. by Paintal, A. S.) (in the press).
- 12 Nakano, J., and Cole, B., *Am. J. Physiol.*, **217**, 222–227 (1969).
- 13 Mills, J. E., Sellick, H., and Widdicombe, J. G., in *Breathing: Hering-Breuer Centenary Symposium* (edit. by Porter, R.), 77–99 (Churchill, London, 1970).
- 14 Smith, A. P., and Cuthbert, M. F., *Br. Med. J.*, **3**, 212–213 (1972).
- 15 Wasserman, M. A., *Pharmacologist*, **16**, 212 (1974).
- 16 DeKock, M. A., Nadel, J. A., Zwi, S., Colebatch, H. J. H., and Olsen, C. R., *J. appl. Physiol.*, **21**, 185–194 (1966).
- 17 Mills, J. E., and Widdicombe, J. G., *Br. J. Pharmac. Chemother.*, **39**, 724–731 (1970).

Multiple sclerosis, Guillain–Barré syndrome and myelin basic protein-specific cellular antibody

MULTIPLE sclerosis (MS) and the Guillain–Barré syndrome (GBS) are demyelinating diseases of unproven aetiology and

pathogenesis, in which infectious and immunological factors have been suggested to have a role^{1–3}. The notion that sensitisation to basic proteins from myelin might be involved has arisen from certain similarities between these human diseases and the autoimmune demyelinating diseases of animals, experimental allergic encephalomyelitis (EAE) and experimental allergic neuritis (EAN)⁴. EAE is caused by sensitisation to the encephalitogenic basic protein from central myelin (CNS-BP) and EAN by sensitisation to peripheral myelin. One of the two basic proteins of peripheral myelin, P2 protein⁵, is considered a likely candidate for the neuritogenic factor⁶. To help determine whether sensitisation to myelin basic proteins is involved in the pathogenesis of MS and GBS, we have used horseradish peroxidase (HRP)-labelled myelin basic proteins^{7,8} to seek evidence of an antibody of this specificity in nervous system plasma cells in these diseases. Our negative findings do not support a pathogenetic role for myelin basic proteins in MS or GBS.

In rabbits with EAE, it has been shown that plasma cells specifically binding a conjugate of CNS-BP and HRP are present in tissue sections of lymph nodes from day 8 until at least day 54 after immunisation^{7,9}. Such cells are also present in early spinal cord lesions^{7,9}, the only time at which they have been sought. Likewise in rabbits with acute EAN, plasma cells specifically binding an HRP conjugate of P2 protein are present in lymph nodes and dorsal root ganglia (DRG)⁸. The binding of these conjugates by plasma cells in these disorders is believed to reflect the presence of intracellular IgG antibody specific for the respective basic proteins¹⁰. The conjugation procedure diminishes somewhat the immunological reactivity of CNS-BP (refs 11, 12), the method does not detect plasmalemmal staining as might be anticipated to occur on CNS-BP reactive lymphocytes (our unpublished observations), and no relationship has been proved between the reactive plasma cells and the pathogenesis of EAE or EAN. Nevertheless, the presence of these basic protein-binding cells serves as an indicator of the presence of immunological reactivity towards the particular antigen, and their presence in the nervous system lesions puts them in a position of potential pathogenetic importance.

There is no conclusive evidence in MS and GBS that sensitisation to myelin basic proteins is involved in pathogenesis. A relationship was, however, recently reported between acute exacerbations of MS and sensitisation to CNS-BP, as measured by a macrophage migration inhibition assay¹³, and sensitisation to peripheral nerve basic proteins has been noted in GBS (refs 14, 15). Whether these sensitisations are primary or secondary features of the diseases is not known. Partly because signs of EAE can be suppressed by injections of CNS-BP and some other compounds¹⁶, trial treatment of MS with CNS-BP or other EAE-suppressive compound is being considered. In MS, IgG antibody is believed to be produced locally within the nervous system¹⁷. MS cerebrospinal fluid contains increased levels of oligoclonal immunoglobulins¹⁸ and increased numbers of IgG-containing cells¹⁹, both of unproven antibody specificity. If plasma cells specifically binding myelin basic proteins were present in MS or GBS, this would support the suggestion that sensitisation to myelin basic proteins may have a role in these diseases.

We have sought cells binding an HRP conjugate of CNS-BP in fresh frozen tissue obtained at autopsy from three chronic cases of MS. Formalin-fixed cryostat sections of nine cerebral MS plaques with adjacent white matter were stained with oil red O and methyl green–pyronin as well as with a light microscopic, peroxidase–CNS-BP conjugate procedure described previously⁷. All the plaques exhibited disease activity, as indicated by the presence of considerable numbers of fat-laden macrophages at the periphery, and four were quite active with lipid macrophages throughout.

Perivascular mononuclear infiltrates and scattered pyronophilic plasma cells, both within the cuffs and free in the parenchyma, were present in seven plaques. Two additional blocks of normal appearing white matter containing scattered pyronophilic mononuclear cells were also studied, as was one lymph node from the case with three of the most active plaques. In addition, we assayed smears of cerebrospinal fluid sediments containing mononuclear cells from four other MS patients and two controls. All these tissues and cells were incubated either with a conjugate of bovine or human CNS-BP, or both. Although these two conjugates both gave positive results in lymph nodes of rabbits immunised with bovine CNS-BP, in none of the MS tissues or cells was specific binding of CNS-BP demonstrable.

Fresh frozen tissue from two fatal cases of GBS was similarly tested, but in this instance for binding of an HRP conjugate of bovine P2 protein⁸. Formalin-fixed cryostat sections of five lumbar and three sacral DRG and two peripheral nerves generally contained mononuclear infiltrates and rare plasma cells, but no specific binding of P2 protein was found. The P2 protein conjugate did reveal positive plasma cells when used with lymph nodes and DRG from rabbits immunised with bovine peripheral myelin.

In contrast to the situation in EAE and EAN, these studies do not support the existence of sensitisation to basic proteins from myelin in either MS or GBS, as measured by cellular anti-basic protein antibody. Serum and cerebrospinal fluid anti-basic protein antibodies also are reported not to be detectable in MS^{20,21}. Since in both EAE and EAN, only a portion of the plasma cells bind basic protein conjugates, sampling of the human material could constitute a limitation of this study. Little is known about the species cross reactivity of P2 protein, but species differences are unlikely to be a factor in the MS results of this study. Both human and bovine CNS-BP were tested, and wide species cross reactivity between human, bovine, rabbit, rat and guinea pig CNS-BP has been demonstrated with this peroxidase procedure (A.B.J., unpublished observations) and by another technique²².

We thank Drs W. W. Tourtellotte, J. M. Powers, A. J. Spiro and F. H. Anlyan for some of the MS and GBS material and Drs E. H. Eylar and S. W. Brostoff for the purified basic proteins. We are particularly grateful to Dr R. D. Terry for his support and encouragement. Ms Norma R. Blum gave technical assistance. This work was supported by grants from the National Multiple Sclerosis Society.

ANNE B. JOHNSON
MAURO C. DAL CANTO*

Department of Pathology (Neuropathology)
and the Rose F. Kennedy Center for Research in
Mental Retardation and Human Development,
Albert Einstein College of Medicine,
Bronx, New York 10461

Received July 9; accepted September 27, 1976.

*Present address: Department of Pathology (Neuropathology), Northwestern University Medical School, Chicago, Illinois 60611.

- 1 Prineas, J. W., in *Handbook of Clinical Neurology*, 9 (edit. by Vinken, P. J., and Bruyn, G. W.), 107-160 (North-Holland, Amsterdam, 1970).
- 2 Waksman, B. H., *New Engl. J. Med.*, **291**, 45-46 (1974).
- 3 Panelius, M., *Med. Biol.*, **53**, 187-194 (1975).
- 4 Alvord, E. C., Jr, in *Handbook of Clinical Neurology*, 9 (edit. by Vinken, P. J., and Bruyn, G. W.), 500-571 (North-Holland, Amsterdam, 1970).
- 5 Brostoff, S. W., Karkhanis, Y. D., Carlo, D. J., Reuter, W., and Eylar, E. H., *Brain Res.*, **86**, 449-458 (1975).
- 6 Carlo, D. J., Karkhanis, Y. D., Bailey, P. J., Wisniewski, H. M., and Brostoff, S. W., *Brain Res.*, **88**, 580-584 (1975).
- 7 Johnson, A. B., Wisniewski, H. M., Raine, C. S., Eylar, E. H., and Terry, R. D., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 2694-2698 (1971).
- 8 Dal Canto, M. C., Johnson, A. B., Raine, C. S., Wisniewski, H. M., and Brostoff, S. W., *J. Immun.*, **113**, 387-394 (1974).
- 9 Johnson, A. B., and Blum, N. R., *J. Histochem. Cytochem.*, **20**, 841 (1972).
- 10 Dal Canto, M. C., Blum, N. R., and Johnson, A. B., *J. Histochem. Cytochem.*, **23**, 452-457 (1975).
- 11 Gonatas, N. K., et al., *Am. J. Path.*, **76**, 529-548 (1974).
- 12 Whitaker, J. N., *J. Histochem. Cytochem.*, **24**, 652-658 (1976).
- 13 Sheremata, W., Cosgrove, J. B. R., and Eylar, E. H., *J. neur. Sci.*, **27**, 413-425 (1976).

- 14 Sheremata, W., Colby, S., Karkhanis, Y., and Eylar, E. H., *Can. J. neur. Sci.*, **2**, 87-90 (1975).
- 15 Abramsky, O., Webb, C., Teitelbaum, D., and Arnon, R., *Neurology*, **25**, 1154-1159 (1975).
- 16 Bernard, C. C. A., Mackay, I. R., Whittingham, S., and Brous, P., *Cell Immun.*, **22**, 297-310 (1976).
- 17 Tourtellotte, W. W., in *Multiple Sclerosis, Immunology and Virology and Ultrastructure* (edit. by Wolfgram, F., et al.), 285-324 (Academic, New York, 1972).
- 18 Norrby, E., and Vandvik, B., *Med. Microbiol. Immunol.*, **162**, 63-72 (1975).
- 19 Sandberg-Wollheim, M., and Turesson, I., *Scand. J. Immun.*, **4**, 831-836 (1975).
- 20 Lisak, R. P., Heinze, R. G., Falk, G. A., and Kies, M. W., *Neurology*, **18**, suppl., 122-128 (1968).
- 21 Schmid, G., Thomas, G., Kempel, K., and Grüniger, W., *Eur. Neurol.*, **12**, 173-185 (1974).
- 22 Guarneri, M., and Cohen, S. R., *Brain Res.*, **100**, 226-230 (1975).

Proposed respiratory 'gating' mechanism for cardiac slowing

THREE of the essential variables concerned in the regulation of systemic cardiovascular performance are cardiac output, blood pressure and the resistance encountered by the blood in flowing through the systemic vascular bed. Cardiac output may be further considered as the product of cardiac stroke volume and heart rate. The latter is under the control of the vagi and sympathetic nerves but the reflex bradycardia produced by vagal activation as a result of increased blood pressure, increased carotid chemoreceptor activity or direct electrical stimulation of the carotid sinus nerve occurs only if the stimulus is delivered during expiration. A sustained stimulus

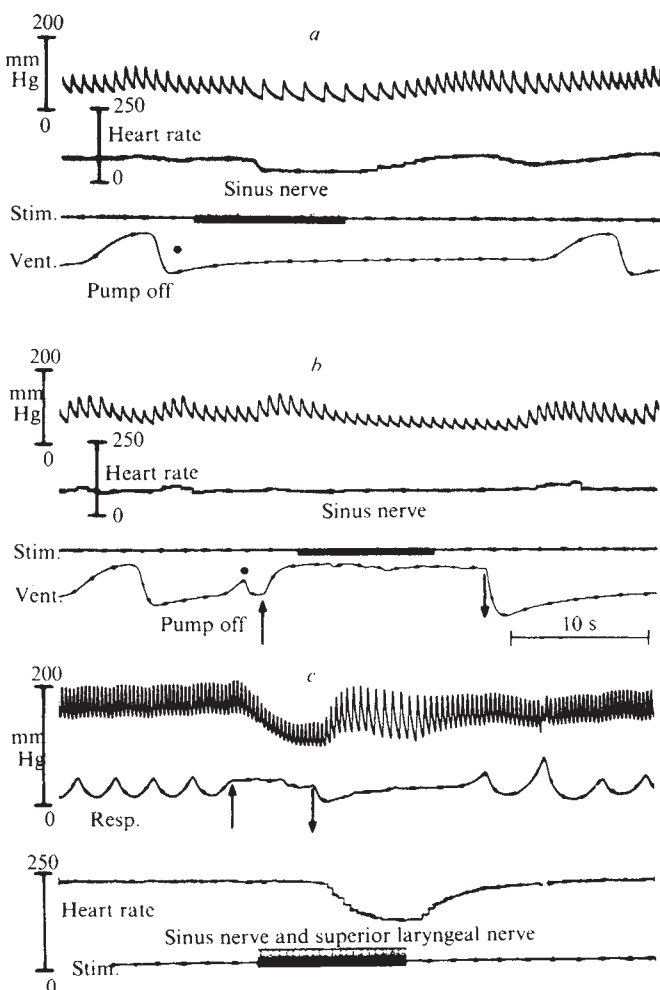


Fig. 1 *a* and *b*, Spinal cord cut at level of first cervical vertebra; cat ventilated with respiratory pump. *a*, Pump switched off and sinus nerve stimulated producing bradycardia; *b*, pump switched off and sinus nerve subsequently stimulated during period of sustained lung inflation (between arrows); no bradycardia. *c*, Intact animal; natural breathing. Sustained lung inflation between arrows. Combined sinus nerve and superior laryngeal nerve stimulation begun during this inflation; only effective in producing bradycardia on deflation of lungs.

which is able reflexly to activate the cardiac vagal efferents will, therefore, produce the pattern of heart rate known as sinus arrhythmia (that is, a slow heart rate regularly interrupted by periods of cardiac acceleration coinciding with inspiration). This association of inspiratory activity with the inhibition of bradycardia is also seen when stimulating certain areas of the brain: areas which produce an inhibition of bradycardia^{2,3}

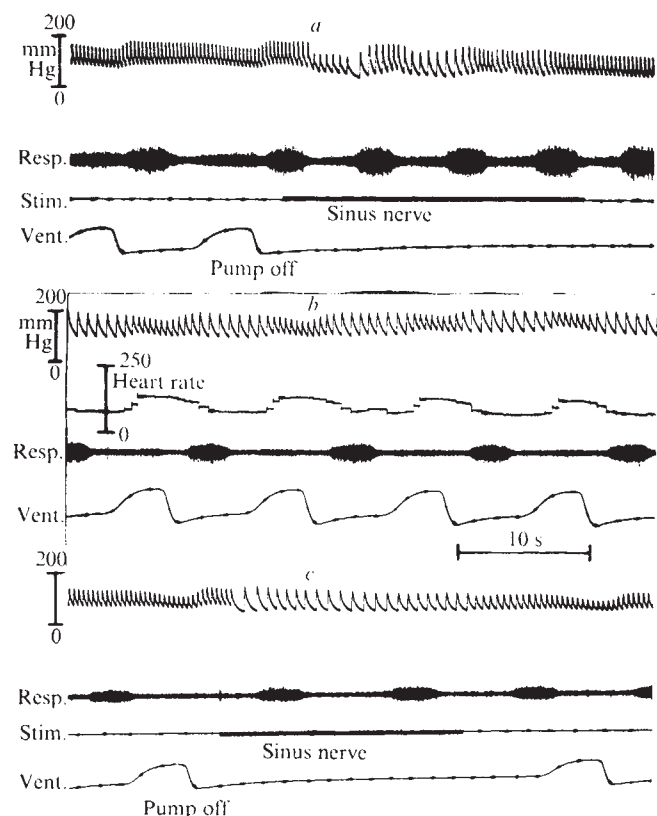


Fig. 2 Spinal cord sectioned at level of first cervical vertebra; cat ventilated with respiratory pump. Central inspiratory drive indicated by recurrent laryngeal electrogram. In *a* the pump is switched off and the sinus nerve stimulated at a strength that increases the intensity and frequency of the central inspiratory drive, which is clearly in phase with the periods of cardiac acceleration of the sinus arrhythmia. (The R_a neurones are then driving the R_b neurones?) In *c* the sinus nerve is stimulated at a reduced strength following a long period of artificial ventilation, and a bradycardia with much less sinus arrhythmia is seen. (The R_b neurones are perhaps less active under these conditions.) In *b* when the lungs are artificially ventilated with an increased stroke volume the periods of cardiac acceleration now correspond with lung inflation and not with the central inspiratory drive. (Possibly in these conditions the R_b neurones, which are powerfully provoked by artificial lung inflation, are insensitive to the natural R_a discharge.)

also produce an increase in respiratory activity, while other areas produce bradycardia and apnoea⁴. A vagal bradycardia unrelated to respiration can in fact only be induced by stimulating the cells of origin in the nucleus ambiguus^{5,6} or the vagus nerve itself. This nucleus lies in the medullary reticular formation ventro-lateral to the nucleus of the tractus solitarius. Since all known afferent stimuli which cause reflex bradycardia converge on the area of the nucleus tractus solitarius where "inspiratory" type neurones abound before passing on to the nucleus ambiguus, it is tempting to postulate that these inspiratory neurones may actually be part of a 'gate' which controls the passage of impulses through this reflex pathway. The rhythmical opening and closing of this gate with respiratory activity would result in sinus arrhythmia provided the gate remains open for a sufficient length of time and that there is an adequate background of vagal tone, which is in itself reflexly engendered. We have experimentally analysed some of the

properties of this proposed respiratory gating mechanism and conclude that it is in fact influenced by both neural inspiratory activity and by lung inflation.

Normally the central inspiratory drive and the resulting lung inflation are almost in phase with the inhibition of cardiac vagal tone. Both the central inspiratory drive and the resulting lung inflation may, however, be abolished by stimulation of the superior laryngeal nerve⁷ which inhibits the neural discharge to both inspiratory and expiratory muscles. If an adequate background level of afferent activity exists, such as is provided by baroreceptor discharge, bradycardia will occur as there is now no hindrance to the afferent impulse traffic to the nucleus ambiguus.

When both aortic and sinus nerves are cut, no bradycardia occurs on stimulation of the superior laryngeal nerve. This shows that stimulation of this nerve does not in itself induce bradycardia. If, in this preparation, the central end of a carotid sinus nerve is stimulated together with the superior laryngeal nerve, profound bradycardia occurs.

Although the superior laryngeal nerve is one of the most powerful means of opening the gate it is not in itself the "master key". If the lungs are kept artificially inflated, not even simultaneous stimulation of the sinus and superior laryngeal nerves produces bradycardia (Fig. 1*c*). As soon as the lungs are deflated this stimulation now provokes bradycardia.

After spinal transection at the level of C1, and with the lungs artificially ventilated, the vagal afferent traffic virtually becomes the only source of information concerning lung volume; in this situation, stimulation of the sinus nerve only elicits bradycardia when artificial respiration is discontinued with the lungs deflated (Fig. 1*a* and *b*). Thus inflation receptors may have a role in the genesis of sinus arrhythmia. It may be seen in Fig. 1*a* that, with the pump switched off, the heart rate is still being modulated by the central respiratory drive, and thus the onset of bradycardia provoked by sinus nerve stimulation is delayed by over 4 s presumably until the central inspiratory drive has temporarily ceased.

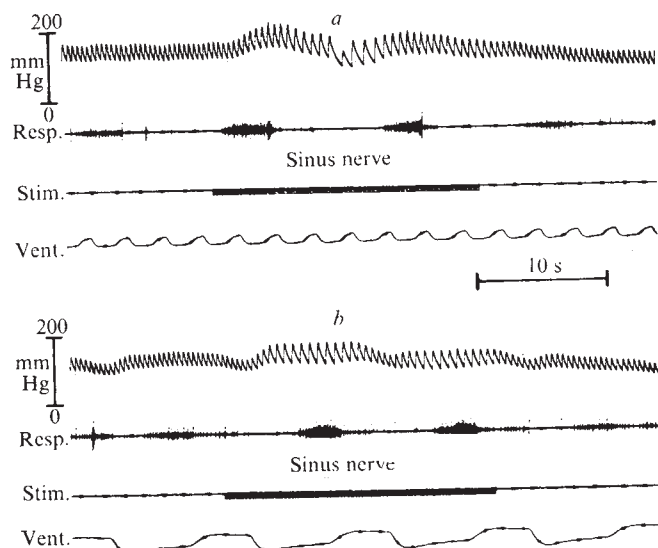


Fig. 3 Intact animal with open chest; ventilation of lungs with pump. Intercoastal CMG to indicate central inspiratory drive. *a*, Pump stroke volume 75 ml; sinus nerve stimulation provokes sinus arrhythmia in which periods of cardiac acceleration correspond with central inspiratory drive and are not in phase with the pump. (R_b are being driven by R_a neurones and are insensitive to small volume high frequency ventilation.) *b*, Pump stroke volume 150 ml; periods of cardiac acceleration in sinus arrhythmia now correspond with lung inflation and are not in phase with central inspiratory drive. (R_b are driven by high volume low frequency lung inflation and are insensitive to natural discharge of R_a neurones.)

It is however also possible to show that in such spinal animals the central respiratory drive can by itself operate this 'gate' in the absence of lung inflation. In Fig. 2a artificial ventilation is temporarily discontinued during which time the sinus nerve is stimulated. In this circumstance sinus arrhythmia can still be observed with its periodic inhibition of bradycardia now in phase with the increased central respiratory drive. When artificial respiration is resumed, with an increased stroke volume bradycardia becomes associated with lung deflation rather than with the expiratory phase of the central respiratory rhythm (Fig. 2b).

Another method of dissociating the dual influence of central drive and lung inflation is to thoracotomise an otherwise intact anaesthetised animal and to ventilate its lungs with a pump. When the respiratory minute volume is adjusted to maintain normal values of pO_2 and pH at low tidal volume and high frequency, the central respiratory rhythm (recorded electromyographically from intercostal muscles) is slower than the prevailing pump frequency: sinus arrhythmia produced by sinus nerve stimulation now occurs in phase with this central rhythm (Fig. 3a). When tidal volume is increased and the rate of pumping slowed, so as to still preserve normal blood gas tensions and pH , the pump drives the central rhythm "out of phase", and now lung deflation occurs during central inspiratory drive: sinus arrhythmia produced by sinus nerve stimulation is now locked to the lung volume changes, with bradycardia in phase with lung deflation (Fig. 3b).

These results suggest the following propositions. (1) The gating mechanism, which may be complex, is probably located in association with both the nucleus tractus solitarius and with the nucleus ambiguus. (2) Complete inhibition of central respiratory drive, such as induced by stimulation of the superior laryngeal nerve, fully opens this 'gate' if, and only if, the lungs are in a state of deflation. (3) The gating mechanism can be operated independently by central drive or by lung inflation. (4) When the lungs are markedly inflated the pulmonary vagal afferent discharge is prepotent in closing the gate.

These considerations strongly suggest that a group of neurones first described by Baumgarten and Kanzow⁸ may qualify as an integral part of this gating mechanism. They described two types of inspiratory neurones in the vicinity of the nucleus tractus solitarius and named them R_a and R_b . The R_a neurones are centrally driven and discharge in phase with the phrenic motor neurones even when the respiratory muscles are paralysed with succinyl choline and both vagi are divided; these R_a neurones are inhibited by lung inflation. The R_b neurones discharge in phase with the R_a , even after succinyl choline and bilateral vagotomy, but unlike the R_a or the phrenic neurones, are stimulated by lung inflation.

R_a neurones do not fulfil the requirements necessary to operate as a gating mechanism as they do not discharge during lung inflation when the gate is shut. R_b neurones, on the other hand, do possess the necessary characteristics. Situated as they are, close to the nucleus tractus solitarius, they could operate in conjunction with the afferent stimulus, for it is known that the "gate" can function within one or two synapses of the afferent inflow⁹. They are independently or simultaneously driven both by central respiratory drive and by lung inflation and are correspondingly active in both situations of "gate" closure. It is possible that their extra discharge induced by powerful lung inflation leads to their becoming temporarily insensitive or 'refractory' to the central command. Such a characteristic might explain the occurrence, under special conditions, of bradycardia coincidental with central inspiratory drive but blocked by lung inflation (Figs 2b, 3b). Similarly after a period of powerful ventilation it is also possible, on suspending artificial ventilation and stimulating the sinus nerve, to induce bradycardia uninterrupted by the tachycardia normally associated with central inspiratory drive (Fig. 2c). Lipski, McAllen and Spyer¹⁰ have analysed the response of R_b neurones to chemoreceptor stimulation in artificially ventilated anaesthetised cats and while finding that the R_b neurones could

be driven by the chemoreceptors during inspiration (with closure of the proposed 'gate'), they also found that the discharge of the R_b neurones during sustained lung inflation was diminished by chemoreceptor stimulation. This might be due to the previously mentioned insensitivity of the R_b neurones to the central inspiratory drive associated with a period of sustained lung inflation and the unmasking of an inhibitory influence of the chemoreceptors on the R_b neurones. Such an influence would be clearly beneficial in the chemoreceptor-induced bradycardia that occurs when efferent respiratory activity is also suspended by simultaneous stimulation of the superior laryngeal nerve¹¹. The presence of ungated baroreceptor units in the vicinity of the nucleus ambiguus⁹ in no way negates the 'gating' hypothesis as the final inhibitory neurone of the gate mechanism may terminate directly on the cell bodies of the nucleus ambiguus.

The R_b neurones, therefore, appear as strong candidates for incorporation in the neuronal organisation of this proposed respiratory 'gate'.

O.U.L. is on leave from Universidade de Sao Paulo, and in receipt of a grant from Fundacao de Amparo a Pesquisa de Sao Paulo, Brasil.

O. U. LOPES
J. F. PALMER

Department of Physiology,
The Middlesex Hospital Medical School,
Cleveland Street,
London, W1P 6DB, UK

Received July 2; accepted October 13, 1976.

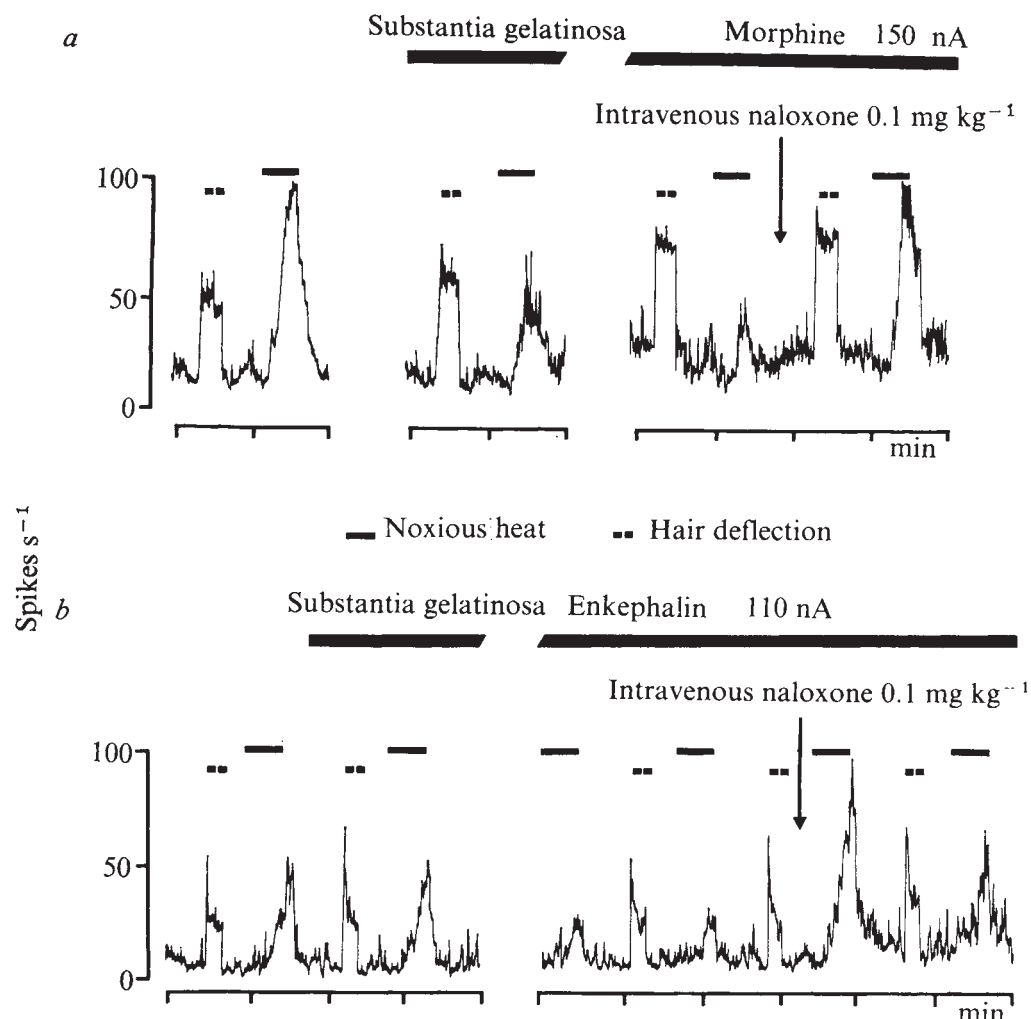
- ¹ Neil, E., and Palmer, J. F., *J. Physiol., Lond.*, **247**, 16P (1975).
- ² Hilton, S. M., *J. Physiol., Lond.*, **165**, 56-57P (1963).
- ³ Abrahams, V. C., Hilton, S. M., and Zbrozyna, A., *J. Physiol., Lond.*, **154**, 491-513 (1960).
- ⁴ Hilton, S. M., and Spyer, K. M., *J. Physiol., Lond.*, **218**, 271-293 (1971).
- ⁵ Gunn, C. G., Sevelius, C., Puiggari, M. J., and Myers, F. K., *Am. J. Physiol.*, **214**, 258-262 (1968).
- ⁶ McAllen, R. M., and Spyer, K. M., *J. Physiol., Lond.*, **244**, 82-83P (1975).
- ⁷ Francois-Franck, *Travaux du Laboratoire de M. Marey II*, 221 (1876).
- ⁸ Baumgarten, R., von Kanzow, E., *Arch. Ital. Biol.*, **96**, 361-373 (1958).
- ⁹ Lipski, J., McAllen, R. M., and Spyer, K. M., *J. Physiol., Lond.*, **251**, 61-78 (1975).
- ¹⁰ Lipski, J., McAllen, R. M., and Spyer, K. M., *J. Physiol., Lond.*, **258**, 115-116P (1976).
- ¹¹ Kordy, M. T., Neil, E., and Palmer, J. F., *J. Physiol., Lond.*, **247**, 24-25P (1975).

Morphine, enkephalin and the substantia gelatinosa

THE pentapeptide enkephalin interacts with opiate receptors of peripheral tissues¹ and with stereospecific opiate receptors of brain homogenates². Ejected from micropipettes in the vicinity of central neurones, it has depressant³⁻⁵ and excitant⁶ effects. These actions, however, cannot be related specifically to the analgesic effects of systematically administered opiates. In electrophoretic experiments, tissue concentrations are unknown and hence even antagonism by electrophoretic naloxone is not necessarily a sufficient test of relevance to the effects of systemic opiates. This report is concerned with the effects of morphine when administered electrophoretically into the substantia gelatinosa. The subsequent changes in the firing of neurones within Rexed laminae IV and V can be related to the effects of this compound given systemically. Furthermore methionine enkephalin had an action similar to that of morphine.

Experiments were performed on cats anaesthetised with α -chloralose. The spinal cord was divided at the thoracolumbar junction. In preliminary experiments morphine (0.07 M) and naloxone (0.1 M, Endo Laboratories) were ejected electrophoretically from the outer barrels of 7-barrel micropipettes and the centre barrels were used for extracellular recording. Drug administration was thus largely into the region of cell bodies. Neurones of spinal laminae IV and V were excited alternately by radiant heat adequate to raise the skin temperature of a hind limb digital pad above 45 °C (a noxious stimulus⁷), and

Fig. 1 The effect of morphine (*a*) and methionine enkephalin amide (*b*) on the firing of lamina IV spinal neurones by a noxious and non-noxious skin stimulus and the reversal of these effects by intravenous naloxone (0.1 mg kg^{-1}). In both *a* and *b* the noxious stimulus was heat to the fourth digital pad of the left hind leg adequate to raise skin temperature above 45°C ; the non-noxious stimulus was deflection of adjacent hairs by a moving air jet. In *a*, 14 min elapsed between the recording of the left and middle groups of responses and 55 min between the middle and right groups. In the latter interval, naloxone, ejected electrophoretically into the substantia gelatinosa, was shown to antagonise the effects of morphine. In *b*, 12 min elapsed between the right and left groups of records.



by deflection of the adjacent hairs by a moving air jet (a non-noxious stimulus). In these conditions morphine failed to reduce selectively the activation of cells by either stimulus, and high ejecting current (80–100 nA) produced abnormalities in action potential amplitude and configuration⁸.

In marked contrast, when morphine was ejected from another micropipette located superficial to the cell body and positioned in the region of the substantia gelatinosa, selective depression of cell responses to the noxious stimulus was observed without effect on those to the non-noxious stimulus (11 to 14 cells). The separation between the tips of the drug-administering and the recording micropipettes varied from 100 to 830 μm . Results from one neurone are illustrated in Fig 1*a*. The middle records show that after 14 min of administration of morphine with an ejecting current of 150 nA, there was approximately a 50% reduction in the response to painful stimuli while the non-pain response was slightly increased. The reduction in the response to painful stimuli by morphine was long lasting, commonly greater than 30 min. This effect of morphine was reversed by naloxone administered electrophoretically in the substantia gelatinosa, and, more significantly, by intravenous naloxone in doses as low as 0.1 mg kg^{-1} . This dose of naloxone reverses the effects of analgesic doses of morphine in the cat⁹ and dog¹⁰ and its effectiveness in our experiments strongly suggests that the concentrations of morphine in the substantia gelatinosa reducing responses to painful stimuli were not inappropriate to those present after systemic morphine. The rapid effects of intravenous naloxone are shown in Fig. 1*a*.

Two samples of enkephalin were ejected from pipettes positioned in the substantia gelatinosa; methionine enkephalin (Calbiochem, 0.015 M, pH 3.5) and methionine enkephalin amide (supplied by Drs H. Niall and G. Tregear, The Howard Florey Institute, Melbourne; 0.020 M, pH 4). Of 17 cells tested, enkephalin ejected into the substantia gelatinosa reduced the responses of 12 to painful stimuli. With six neurones this reduction occurred in the absence of any effect on responses to painless stimulation or on spontaneous firing, with five the predominant effect was a reduction in the response to painful stimuli with a smaller reduction in the response to painless ones, and with one cell all three firing patterns were reduced in parallel. In the latter case the separation between the electrode tips was the smallest used. More consistent effects were observed with methionine enkephalin amide (10 of 11 cells tested) than with methionine enkephalin (2 of 6 cells). Figure 1*b* shows the selective reduction of the responses to painful stimulation of a lamina IV neurone after 13 min of ejection of methionine enkephalin amide (110 nA) in the substantia gelatinosa. In contrast to morphine, recovery from the effects of enkephalin was rapid, being complete within 10 min of the termination of ejection.

The reduction of responses to painful stimulation by enkephalin was fully reversed in 5 of 6 animals by a low dose of intravenous naloxone (0.1 mg kg^{-1} in four cats, and 0.3 mg kg^{-1} in one). The effect of naloxone was rapid as illustrated in Fig. 1*b*. All observed effects of enkephalin were reversed by naloxone—when response to non-painful stimulation and spontaneous firing were depressed by enkephalin this depression was also reversed by intravenous

naloxone. With one neurone, intravenous naloxone 0.1 mg kg^{-1} partially reversed the effects of enkephalin; this effect was not increased by additional doses up to a total of 1.2 mg kg^{-1} .

These results suggest that a receptor for morphine in the substantia gelatinosa is indeed relevant to the reduction, by systemic opiates, of pain with minimal effect on other sensations¹¹. The substantia gelatinosa contains the endings of primary afferent fibres, dendrites of deeper neurones, and small neurones which project only to other parts of the substantia gelatinosa. Axo-axonic synapses are abundant¹². Autoradiographic¹³ and binding studies¹⁴ have shown this to be a region of high opiate binding, but further experiments are needed to determine the fine structural localisation of the morphine receptors of the substantia gelatinosa and their relevance to primary afferent fibres and interneurons associated with painful stimuli. The methods used in the present experiments offer a means for determining the possible physiological role of enkephalin.

A. W. DUGGAN
J. G. HALL
P. M. HEADLEY

Department of Pharmacology,
Australian National University,
Canberra, ACT, Australia

Received August 31; accepted October 4, 1976.

- 1 Hughes, J., *Brain Res.*, **88**, 295–308 (1975).
- 2 Simantov, R., Kuhar, M. J., Pasternak, G. W., and Snyder, S. H., *Brain Res.*, **106**, 189–197 (1976).
- 3 Bradley, P. B., Briggs, I., Gayton, R. J., and Lambert, L. A., *Nature*, **261**, 425–426 (1976).
- 4 Gent, J. P., and Wolstencroft, J. H., *Nature*, **261**, 426–427 (1976).
- 5 Hill, R. G., Pepper, C. M., and Mitchell, J. F., *Nature*, **262**, 604–606 (1976).
- 6 Davies, J., and Dray, A., *Nature*, **262**, 603–604 (1976).
- 7 Beck, P. W., Handwerker, H. O., and Zimmermann, M., *Brain Res.*, **67**, 373–386 (1974).
- 8 Duggan, A. W., and Curtis, D. R., *Neuropharmac.*, **11**, 189–196 (1972).
- 9 Jurna, I., and Grossmann, W., *Exptl Brain Res.*, **24**, 473–484 (1967).
- 10 McCane, T. K., and Martin, W. R., *Int. J. Neuropharmac.*, **6**, 325–327 (1967).
- 11 Kreuger, H., in *The Alkaloids* (ed. Manske, R. H. F.), pp. 2–78 (Academic, New York, 1955).
- 12 Réthelyi, M., and Szentágothai, J., *Exptl Brain Res.*, **7**, 258–274 (1969).
- 13 Pert, C. B., Kuhar, M. J., and Snyder, S. H., *Life Sci.*, **16**, 1849–1854 (1975).
- 14 Lamotte, C., Pert, C. B., and Snyder, S. H., *Brain Res.*, **112**, 407–412 (1976).

Intraventricular Met⁵-enkephalin causes unexpected lowering of pain threshold and narcotic withdrawal signs in rats

PEPTIDES with opiate properties have been demonstrated in brain^{1–3} and pituitary^{4–7}. Goldstein⁸ has postulated that sustained low-intensity pain might promote the central mobilisation of endogenous opioid as part of an adaptive response to noxious stimuli which cause suffering but do not threaten survival. We have examined the effects of intraventricular infusion of an endogenous opioid peptide, methionine-enkephalin⁹ (Met⁵-enkephalin), on responses to a sustained mildly noxious stimulus⁹ in rats. We expected Met⁵-enkephalin to attenuate responsiveness to the noxious stimulus, as has been reported¹⁰ for its effects on acute pain. On the contrary in our experiments, however, it seems to have increased responsiveness, and moreover to have induced behaviour typical of opiate withdrawal.

Thirteen male Sprague–Dawley albino rats, 180–200 g, were implanted under pentobarbitone anaesthesia (Nembutal, 50 mg kg⁻¹) stereotactically with chronic intraventricular cannulae in the right lateral ventricle (De Groot¹¹: A-3.2, L-5.0, V-5.1) according to the technique of Rezek and Havlicek¹². Correct placement of cannulae and success of infusion were confirmed histologically. Rats were first tested (test 1) 8 d after cannula implantation. They were given intraventricular infusions of either 10 μl saline over 65 s (saline control group of test 1) or of 100 μg Met⁵-enkephalin (Beckman Bioproducts) in an identical volume and rate of saline infusion. At 1.0 min after the end of

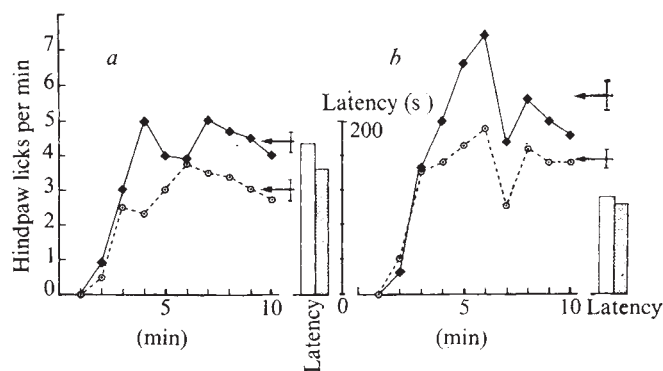


Fig. 1 *a*, Effect of Met⁵-enkephalin (stippled column and $\blacklozenge$) via right lateral ventricular infusion on hindpaw licks in rats kept on 44.5°C hotplate for 10 min (test 1 of cross-over test). For a given group, each point on graphs indicates average number of hindpaw licks per animal observed over the minute preceding the plotted time. Height of arrowed horizontal lines above abscissa indicates hindpaw licks per min over last 7 min of the 10-min test; their vertical lines show standard errors. Six rats were infused with Met⁵-enkephalin, seven with saline (open column and $\circ$) as controls. Latency is time in s when first hindpaw lick was observed after rat was placed on hotplate; height of latency bar represents mean for each group. *b*, Test 2 of crossover test. Rats in Met⁵-enkephalin group ($n = 5$) were all taken from those in the saline group of test 1; rats in the saline group ($n = 4$) were all taken from those in Met⁵-enkephalin group of test 1. Other details as in *a*.

infusion the animals were placed within a vented transparent cylinder (48 cm high $\times$ 20 cm in diameter with top open) on a hotplate at 44.5°C (ref. 9). There was a significantly ($P \leq 0.01$) higher frequency of hindpaw licking in the Met⁵-enkephalin-infused group than in the saline controls, evaluated over the last 7 min of the 10-min test (Fig. 1*a*). Significant differences did not occur between hindpaw licking frequency in the two groups over the first 3 min, nor between latencies to the first hindpaw lick (Fig. 1*a*).

All animals with cannulae still intact after 5 d were retested (test 2) in crossover fashion; those which had received saline in test 1 were given infusions of Met⁵-enkephalin, and vice versa. Again, the Met⁵-enkephalin-infused group exhibited significantly ($P \leq 0.02$) more hindpaw licking than did the saline-infused rats (Fig. 1*b*), thus confirming results of the first test. Mean latency to first hindpaw lick was shorter in the Met⁵-enkephalin-infused rats in test 2 (Fig. 1*b*) than in the same animals during infusion with saline in test 1 (Fig. 1*a*). The difference was significant ($P \leq 0.05$) by single-tailed *t*-test for paired comparisons.

In both tests, all the animals infused with Met⁵-enkephalin exhibited signs of narcotic withdrawal¹³, that is: wet dog shakes, rearing, sniffing, restless exploring activity and increased defaecation, beginning at 1–3 min after the start of infusion. In some animals these signs were particularly severe. None of the rats showed these signs when infused with saline in either test.

Six of the rats used in tests 1 and 2 were retested on the hotplate, 7 d after test 2, with infusion of 2.0 μg naloxone hydrochloride (Endo Laboratories) in 10 μl saline/65 s. The animals showed significantly ($P \leq 0.05$) more hindpaw licking than did the saline-infused animals of either test 1 or 2 but displayed no signs of narcotic withdrawal. Retesting three of these animals with 15.0 μg naloxone 10 d later gave a similar result.

The hotplate test used here differs from the conventional hotplate test for analgesia in that the surface temperature is low enough to permit exposure of the experimental animal to a 10-min sustained mildly noxious environment in a demarcated space. It therefore permits observations to be made on the effects of drugs on the behavioural responses to such exposure; hindpaw licking is a prominent feature of those responses in rats. We believe it justified to regard hindpaw licking as an indication of pain, since it is readily distinguishable from

normal grooming activity and is much reduced by morphine given either intraperitoneally⁹ or intraventricularly. The effects of intraventricular Met⁵-enkephalin in causing an apparent increase in perception of pain, as well as signs of opiate narcotic withdrawal, were totally unexpected.

Leucine-enkephalin (Leu⁵-enkephalin) (Peninsula Laboratories) was tested in a separate series of experiments with 18 rats, also chronically implanted with intraventricular cannulae. Three groups of six rats were first tested after infusion of saline. In a second test 6 animals were given saline, morphine sulphate (10 µg), or Leu⁵-enkephalin (100 µg) (Fig. 2). It can be seen that morphine and Leu⁵-enkephalin significantly diminished hindpaw licking in response to the hotplate. Thus, Leu⁵-enkephalin, in contrast to Met⁵-enkephalin, exhibits analgesic action like that of morphine.

Our findings with Met⁵-enkephalin may be explained by three main factors: (1) inability of the pentapeptide opiate to reach sites of opiate analgesic action in caudal portions of the ventricular system¹⁴; such failure could be due to rapid destruction of Met⁵-enkephalin by brain peptidases¹⁵ and, perhaps, to poor lipid solubility; (2) excitation, by Met⁵-enkephalin, of sites of "paradoxical" opiate action^{16,17}; (3) the conditions of our hotplate test, which may induce the mobilisation and release of endogenous opiate-like substances in the brain, their target being stereospecific opiate receptor sites concerned with the attenuation of pain perception.

It is conceivable that among the released endogenous opioids there exists an unidentified opiate substance which is a more potent opiate agonist than Met⁵-enkephalin. After several minutes of stimulation by such an agonist, the system may adapt to, and thus become "dependent" on, the potent opiate; very rapid acquisition of opiate narcotic tolerance and physical dependence has been seen in several studies¹⁸⁻²⁰. The intraventricular infusion of Met⁵-enkephalin in such an opiod-

Leu⁵-enkephalin acts like morphine is compatible with *in vitro* data showing it to be a 'purer' agonist than Met⁵-enkephalin⁷. It may be that such mixed action of any opiate *in vivo* is simply the expression of the drug's relative ability both to displace endogenous opiate from opiate receptor sites and to act there itself. Failure of intraventricular naloxone (which aggravated pain but produced no signs of narcotic withdrawal) to behave identically with Met⁵-enkephalin might be due to differences in affinity between these substances for opiate receptors at sites differentially involved in the two behaviours. Aggravation of pain with parenteral naloxone at low doses has already been described^{21,22}.

Various endogenous molecular species^{1-7,23,24} may interact with the opiate receptor in response to noxious stimuli. Prominent among these is the C-fragment of β -lipotropin²⁵. The C-fragment molecule is 35 times as potent as Met⁵-enkephalin in displacing ³H-dihydromorphine; it has been reported to cause prolonged analgesia after infusion into the third ventricle of cat, and to show an analgesic potency, on a molar basis, 200 times that of morphine²⁵. Our present findings with intraventricular Met⁵-enkephalin and naloxone suggest that several endogenous opioids may be competing at multiple sites when mobilised during painful stimulation.

This work is supported by the MRC and NMUD/Health and Welfare Canada; L.L. is a postdoctoral fellow of the University of Manitoba Research Board and F.S.L. is an Associate of the MRC.

L. LEYBIN

C. PINSKY*

F. S. LABELLA

Department of Pharmacology and Therapeutics

V. HAVLICEK

M. REZEK

Department of Physiology,
University of Manitoba
Faculty of Medicine,
770 Bannatyne Avenue,
Winnipeg, Manitoba R3E 0W3, Canada

Received June 7; accepted October 4, 1976.

*To whom reprint requests should be addressed.

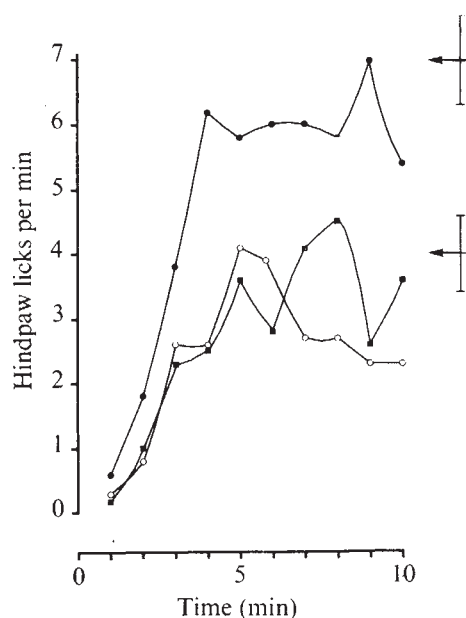


Fig. 2 Effect of Leu⁵-enkephalin (■), morphine (○) or saline (●) on rats subjected to a hotplate. Other details as in Fig. 1.

adapted brain would result in the displacement of the more potent endogenous opiate-like substance, with the consequent signs of narcotic withdrawal and hypernociception that we observed in our experiments. Studies⁹ on isolated organs *in vitro* have shown that Met⁵-enkephalin has predominantly opiate agonist properties. Nevertheless, its sodium sensitivity in opiate receptor binding studies⁷ suggests that Met⁵-enkephalin has at least some measure of opiate antagonist action, and the *in vivo* results described here indicate that Met⁵-enkephalin behaves as a mixed opiate agonist-antagonist. Our finding that

- Hughes, J., *Brain Res.*, **88**, 295-308 (1975).
- Terenius, L., and Wahlstrom, A., *Acta pharmac. tox.*, **35**, Suppl. I, 55 (1974).
- Hughes, J., et al., *Nature*, **258**, 577-579 (1975).
- Teschemacher, H., Opheim, K. E., Coz, B. M., and Goldstein, A., *Life Sci.*, **16**, 12, 1771-1776 (1975).
- LaBella, F. S., Dular, R., Leybin, L., and Pinsky, C., *Abstracts, 58th Mtg. of The Endocrine Society* (in the press).
- Guillemin, R., Ling, N., and Burgus, R., *C. r. hebdom. Séanc. Acad. Sci., Paris, Ser. D.*, **282**, 783-785 (1976).
- Simantov, R., and Snyder, S. H., *Life Sci.*, **18**, 781-788 (1976).
- Goldstein, A., in *Can. Fed. Biol. Soc., 17th Ann. Mtg.* (1974).
- Pinsky, C., Koven, S. J., and LaBella, F. S., *Life Sci.*, **16**, 12, 1785-1786 (1975).
- Belluzzi, J. D., et al., *Nature*, **260**, 625-626 (1976).
- De Groot, J., *Verhandelingen Der Koninklijke Nederlandse Akademie Van Wetenschappen, AFD. Natuurkunde*, **2**, 3-40 (1959).
- Rezek, M., and Havlicek, V., *Physiol. Psychol.*, **3**, 263-264 (1975).
- Wei, E., Loh, H. H., and Way, E. L., *J. Pharmac. exp. Ther.*, **184**, 2, 398-403 (1973).
- Herz, A., Albus, K., Metys, J., Schubert, P., and Teschemacher, H., *Neuropharmacology*, **9**, 539-551 (1970).
- Hughes, J., Smith, T., Morgan, B., and Fothergill, L., *Life Sci.*, **16**, 1753-1758 (1975).
- Jacquet, Y. F., and Lajtha, A., *Science*, **182**, 490-492 (1973).
- Jacquet, Y. F., and Lajtha, A., *Science*, **185**, 1055-1057 (1974).
- Cheney, D. L., and Goldstein, A., *Nature*, **232**, 477-478 (1971).
- Kosersky, D. S., Harris, R. A., and Harris, L. S., *Eur. J. Pharmac.*, **26**, 122-124 (1974).
- Eidelberg, E., and Erspamer, R., *Arch. int. Pharmacodyn.*, **211**, 58-63 (1974).
- Jacob, J. J., Tremblay, E. C., and Colombel, M. C., *Psychopharmacologia*, **37**, 217-223 (1974).
- Gigliotti, O., and Pinsky, C., *RODA Summer Scholarships Abstracts*, 91 (Health and Welfare Canada Publ., Ottawa, 1974).
- Craig, C. R., *J. Pharmac. exp. Ther.*, **164**, 371-379 (1968).
- Bradbury, A. F., Smyth, D. G., Snell, C. R., Birdsall, N. J. M., and Hulme, E. C., *Nature*, **260**, 793-795 (1976).
- Feldberg, W. S., and Smyth, D. G., *J. Physiol., Lond.* (in the press).

Enkephalin inhibits firing of myenteric neurones

ENDOGENOUS ligands for the opiate receptor have recently been described. The first to be characterised were methionine-enkephalin and leucine-enkephalin, pentapeptides isolated from porcine brain by Hughes *et al.* and subsequently synthesised¹. The enkephalins are also found in high concentrations in the myenteric plexus of the guinea-pig ileum^{2,3}, a neuronal network which is widely used as a model in investigations of narcotic action⁴. In the myenteric plexus, narcotic analgesics inhibit the neuronal firing which can be recorded with a glass suction electrode^{5,6}. This action is stereospecific, is reversed by narcotic antagonists such as naloxone and is not dependent upon the presence of any synaptic input to the neurones⁶. The endogenous presence of enkephalin in brain prompted an examination of its effects when applied by iontophoresis to units whose activity was recorded extracellularly⁷⁻¹⁰. We have adopted an alternative approach in which known concentrations of enkephalin are applied to isolated ganglia of the myenteric plexus whilst recording neuronal activity. The results indicate that low concentrations of the enkephalins act on the myenteric neurones to inhibit their firing.

Ganglia of the myenteric plexus, adherent to the longitudinal muscle layer, were perfused in a Krebs saline solution. Activity was recorded from single neurones with a glass suction electrode of 30–50- μ m tip diameter. The myenteric neurones are not spontaneously active within the isolated ganglia, but recently we have shown that the firing recorded with a suction electrode is initiated in the cell soma probably as a consequence of mechanical contact with the recording electrode (North and Williams, in prepara-

tion). In these conditions recordings can be made from single neurones for periods of several hours. The present results are based on recordings from 80 units in 20 guinea pigs. Both methionine-enkephalin and leucine-enkephalin caused an immediate inhibition of neuronal firing (Fig. 1). The depression of spike firing was dose-related; neuronal firing was often completely stopped by 100 nM Met⁵-enkephalin, and markedly inhibited by 10 nM. The firing rate increased immediately on washing with drug-free Krebs solution; in the majority of neurones the washout was associated with a transient but marked "rebound" increase in the rate of discharge (Fig. 1).

Met⁵-enkephalin was approximately five times more potent than Leu⁵-enkephalin when tested on the same unit (Fig. 1). Met⁵-enkephalin was five to ten times more potent than morphine or normorphine. When Met⁵-enkephalin is used to inhibit the nerve-mediated contractile response of the longitudinal muscle layer, it is approximately equipotent with morphine¹¹. However, in those experiments there is a relatively large piece of tissue whereas in our experiments there are only a few isolated ganglia. It is possible that the potency of enkephalin appears to be less in the experiments on the complete longitudinal muscle strip because a large proportion of the dose administered is bound nonspecifically and subsequently destroyed by peptidase. Further support for this notion derives from experiments in which we applied enkephalin for periods of several minutes. When the whole strip is used, the contractile response of the muscle begins to recover after about 1 min despite the continued presence of the enkephalin. However, the firing rate of a single neurone remained depressed for as long as 20 min when the tissue was perfused by a solution containing Met⁵-enkephalin (Fig. 1). There was no evidence of tachyphylaxis. The action of enkephalin was reversed or prevented by a tenfold lower concentration of naloxone (Fig. 2) this concentration of naloxone applied alone was

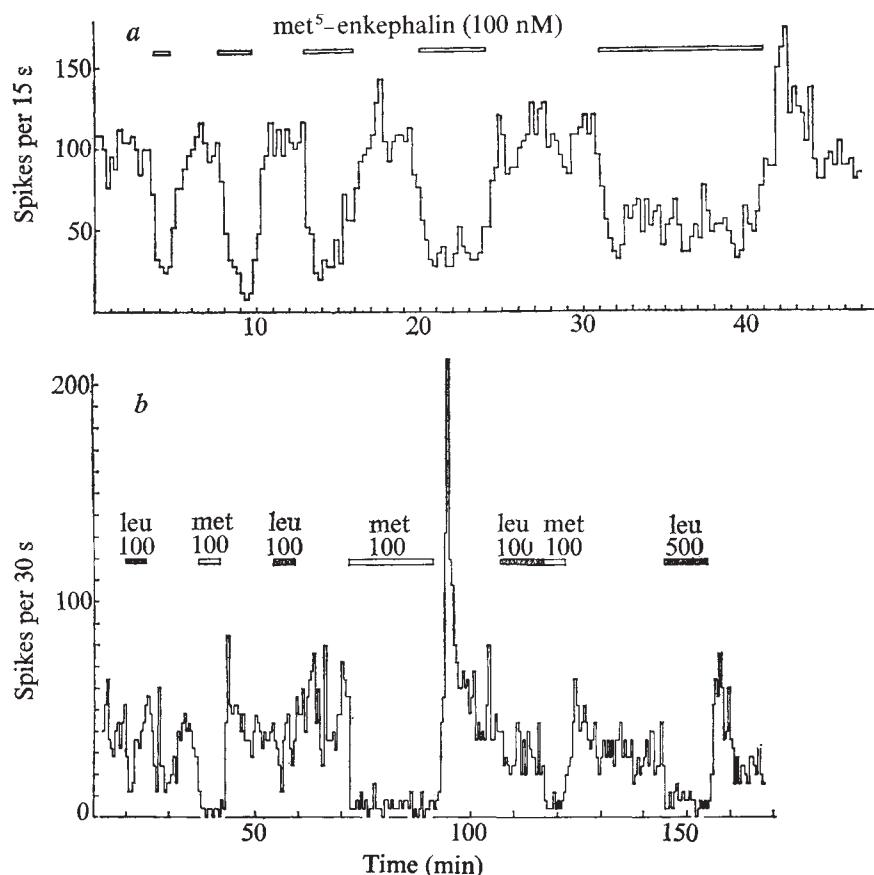
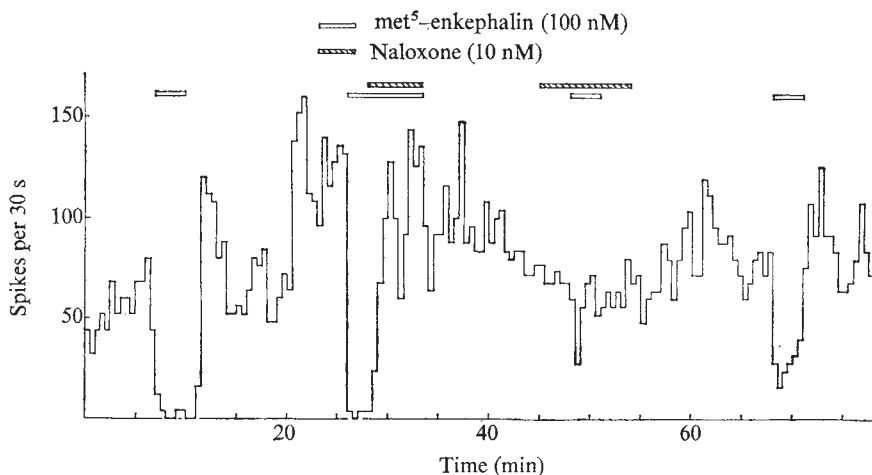


Fig. 1 Inhibition of firing of myenteric neurones by enkephalin. An isolated ganglion of the myenteric plexus was perfused with Krebs solution at 37 °C (solution composition (mmol l⁻¹): NaCl, 117; KCl, 4.7; CaCl₂, 2.5; MgCl₂, 1.2; NaHCO₃, 25; NaH₂PO₄, 1.2; glucose, 11.5; gassed with 95% O₂/5% CO₂). During the periods indicated by the horizontal bars the solution which perfused the tissue contained Met⁵-enkephalin or Leu⁵-enkephalin. *a*, Periods of exposure to Met⁵-enkephalin were 1, 2, 3, 4 and 10 min. In each case there was a marked inhibition of neuronal firing which reversed rapidly when the enkephalin application was stopped. *b*, A similar experiment on a different unit. Leu⁵-enkephalin (100 nM) had only a small and variable effect on cell firing, whereas Met⁵-enkephalin (100 nM) inhibited firing almost completely. The effect of Met⁵-enkephalin (100 nM) was similar to that of Leu⁵-enkephalin (500 nM). Notice in both experiments that there was no evidence of tachyphylaxis during exposures as long as 10 or 20 min. In these and other experiments, the "rebound" increase in firing rate when the enkephalin was washed out was more pronounced with longer periods of exposure.

Fig. 2 Effect of Met⁵-enkephalin and naloxone on the firing rate of a single myenteric neurone. During the periods indicated by the open and hatched bars, the solution which perfused the tissue contained Met⁵-enkephalin (100 nM) and naloxone (10 nM) respectively. Application of Met⁵-enkephalin for 3 min produced a complete inhibition of firing. This inhibition was rapidly reversed by changing to a solution which contained both Met⁵-enkephalin and a tenfold lower concentration of naloxone. In other experiments (for example Fig. 1) we have shown that the firing rate remains depressed by Met⁵-enkephalin even during prolonged applications. Naloxone itself was without significant effect on the firing rate, but it almost completely prevented the action of Met⁵-enkephalin. The final application of Met⁵-enkephalin to this neurone was probably somewhat reduced by the residual presence of some naloxone.



without effect on the firing rate. This finding substantiates the view that the enkephalin is acting on opiate receptors.

The technique which we have used offers certain advantages over the iontophoretic application of enkephalin *in vivo*. The precise concentration of enkephalin at the neurone is known, and degradation by tissue peptidase is largely avoided. Furthermore, as the cell firing is not dependent on synaptic input (ref. 6 and R.A.N. and J.T.W., in preparation), the enkephalins must be acting directly upon the neurone whose activity is recorded. The inhibition of firing by opiates was postulated to be due to a membrane hyperpolarisation⁶, and such an effect of morphine has recently been demonstrated by intracellular recording^{12,13}. The inhibition of neuronal firing by enkephalin may also be based on a membrane hyperpolarisation (R.A.N., unpublished). Inhibitory synaptic potentials have not been recorded in the myenteric plexus, and the role of enkephalin in this tissue remains obscure. On the other hand, if enkephalin inhibits the firing of central neurones by a similar mechanism then its candidacy as inhibitory transmitter would be supported.

We thank Dr B. A. Morgan, Reckitt and Colman Ltd, for a gift of Leu⁵- and Met⁵-enkephalin, Dr E. L. May for a gift of normorphine and Endo Laboratories for a gift of naloxone. The work was supported by a US Public Health Service grant.

R. ALAN NORTH

JOHN T. WILLIAMS

Neurophysiology Laboratory,
Department of Pharmacology,
Loyola University Stritch School of Medicine,
Maywood, Illinois 60153

Received August 31; accepted October 14, 1976.

- ¹ Hughes, J., Smith, T. W., Kosterlitz, H. W., Fothergill, L. A., Morgan, B. A., and Morris, H. R., *Nature*, **258**, 577-579 (1975).
- ² Smith, T. W., Hughes, J., Kosterlitz, H. W., and Sosa, R. P., in *Opiates and Endogenous Opioid Peptides*, 57-62 (North-Holland, Amsterdam, 1976).
- ³ Elde, R., Hökfelt, T., Johansson, O., and Terenius, L., *Neuroscience*, **1**, 349-351 (1976).
- ⁴ Kosterlitz, H. W., and Waterfield, A. A., *A. Rev. Pharmacol.*, **15**, 29-45 (1975).
- ⁵ Sato, T., Takayanagi, I., and Takagi, K., *Japan. J. Pharmacol.*, **23**, 665-671 (1973).
- ⁶ Dingle, R., and Goldstein, A., *J. Pharmacol. exp. Ther.*, **196**, 97-106 (1976).
- ⁷ Bradley, P. B., Briggs, I., Gayton, R. J., and Lambert, L. A., *Nature*, **261**, 425-426 (1976).
- ⁸ Gent, J. P., and Wolstencroft, J. H., *Nature*, **261**, 426-427 (1976).
- ⁹ Davies, J., and Dray, A., *Nature*, **292**, 603-604 (1976).
- ¹⁰ Hill, R. G., Pepper, C. M., and Mitchell, J. F., *Nature*, **262**, 605-606 (1976).
- ¹¹ Waterfield, A. A., Hughes, J., and Kosterlitz, H. W., *Nature*, **260**, 624-625 (1976).
- ¹² North, R. A., *Neuropharmacology*, **15**, 719-721.
- ¹³ North, R. A., and Tonini, M., in *Opiates and Endogenous Opioid Peptides*, 205-212 (North-Holland, Amsterdam, 1976).

Gonadotropin-releasing hormone surge in pro-oestrous rats

It has generally been accepted that the pre-ovulatory surge of luteinising hormone (LH) is caused by an increased release of gonadotropin-releasing hormone (GnRH) from nerve terminals in the median eminence. So far, however, estimations of the concentrations of GnRH in hypophyseal portal vessel blood by bioassay¹ or radioimmunoassay^{2,3} have failed to demonstrate a surge of GnRH before or during the spontaneous surge of LH. The anaesthetics used (urethane^{1,3} and sodium pentobarbitone²) block ovulation, and, therefore, are likely to block or truncate a surge of GnRH, if it occurs. We have re-examined this problem using Althesin (Glaxo, Middlesex) an anaesthetic which consists of 9 mg alphaxalone (3 α -hydroxy-5 α -pregnane-11,20-dione) and 3 mg alphadolone acetate (21-acetoxy-3 α -hydroxy-5 α -pregnane-11,20-dione) per ml of isotonic aqueous vehicle and show that it does not block the spontaneous LH surge or ovulation. Previous studies on the cat⁴ suggested that, although it can provide adequate analgesia, Althesin does not interfere with various forebrain functions.

All experiments were carried out on female Wistar rats, 200-300 g body weight, which were maintained under controlled lighting (lights on 0500-1900) and temperature (22 °C), and exhibited regular 4-d oestrous cycles. The animals were given free access to Diet 41B (F. Dixon & Sons, Ware) and tapwater. A dose of 0.5-0.6 ml Althesin per 100 g body weight, administered intraperitoneally, produced adequate analgesia (no somatomotor response to painful stimuli) for 2.0-3.5 h. Supplementary doses of 0.25-0.5 ml could be given at 2.0-h intervals without risk of fatality.

To test the effect of Althesin on ovulation and LH release, 14 rats were anaesthetised at 1330 on the day of pro-oestrus, and blood samples (0.3 ml) were taken from the external jugular vein either at 1800 ($n = 8$) or 5, 30 and 60 min ($n = 6$) after injection. Inspection of the oviducts on the following morning (expected day of oestrus) revealed that ovulation occurred in all the animals (mean $\pm$ s.e.m. ova count per animal = 8.6 ± 1.0). The mean $\pm$ s.e.m. concentration of LH, estimated by radioimmunoassay^{5,6}, in the samples taken at 1800 was 25.0 ± 8.8 ng NIH-LH-S18 ml⁻¹. Although lower than those in unanaesthetised animals or in animals anaesthetised immediately before blood withdrawal⁶⁻⁸, these results showed that, in contrast to sodium pentobarbitone and urethane, Althesin administered before the 'critical period' of pro-oestrus⁹ did not block the spontaneous surge of LH or ovulation. The plasma LH concentrations of the blood samples taken 5, 30 and 60 min after Althesin injection were 3.4 ± 0.5 , 3.1 ± 0.3 and 3.4 ± 0.3 ng ml⁻¹ respectively, indicating that Althesin does

Table 1 Mean ($\pm$ s.e.m.) concentrations of GnRH in pituitary stalk blood and volumes of collections

Group	Stage and time (h) of cycle	No. of animals	No. of samples in which GnRH detectable*	Conc. GnRH (pg ml ⁻¹)	Blood volume (μ l per 30 min)
1	Metoestrus 0900–1100	23	20	25 $\pm$ 3	513 $\pm$ 56
2	Metoestrus 1600–1830	22	21	34 $\pm$ 7	301 $\pm$ 40
3	Dioestrus 0900–1100	26	18	23 $\pm$ 3	475 $\pm$ 40
4	Dioestrus 1600–1830	23	20	20 $\pm$ 2	363 $\pm$ 28
5	Pro-oestrus 0800–0930	16	11	26 $\pm$ 4	382 $\pm$ 41
6	Pro-oestrus 0930–1030	18	17	31 $\pm$ 3	605 $\pm$ 123
7	Pro-oestrus 1030–1130	21	21	27 $\pm$ 2	461 $\pm$ 59
8	Pro-oestrus 1130–1500	52	43	24 $\pm$ 2	371 $\pm$ 30
9	Pro-oestrus 1500–1600	16	13	49 $\pm$ 11	296 $\pm$ 72
10	Pro-oestrus 1600–1630	12	12	77 $\pm$ 30	448 $\pm$ 112
11	Pro-oestrus 1630–1700	14	14	80 $\pm$ 38	418 $\pm$ 80
12	Pro-oestrus 1700–1730	15	15	85 $\pm$ 34	384 $\pm$ 70
13	Pro-oestrus 1730–1800	14	13	151 $\pm$ 75	327 $\pm$ 63
14	Pro-oestrus 1800–1830	9	9	121 $\pm$ 77	264 $\pm$ 50
15	Pro-oestrus 1830–2000	16	14	45 $\pm$ 4	556 $\pm$ 91
16	Pro-oestrus 2000–2230	27	21	33 $\pm$ 3	482 $\pm$ 65
17	Pro-oestrus 2230–2400	18	9	37 $\pm$ 8	440 $\pm$ 65
18	Oestrus 0000–0200	22	15	66 $\pm$ 13	389 $\pm$ 50
19	Oestrus 0200–0400	18	8	35 $\pm$ 7	319 $\pm$ 37
20	Oestrus 0900–1100	22	20	26 $\pm$ 3	395 $\pm$ 39
21	Oestrus 1600–1830	34	33	18 $\pm$ 1	419 $\pm$ 45

*For definition see text.

Significance of differences between means as determined by analysis of variance and multiple range test:

 $P < 0.05$: group 10 compared with groups 4 and 21; group 11 compared with groups 3, 4, 8 and 21; group 12 compared with groups 1, 3–5, 7, 8, 20 and 21; group 14 compared with group 18. $P < 0.01$: group 13 compared with group 12. $P < 0.005$: group 13 compared with groups 10 and 11; group 14 compared with groups 9, 15 and 17. $P < 0.001$: group 13 compared with all groups except 10–12 and 14; group 14 compared with groups 1–8, 16 and 19–21.

not stimulate LH release. A possible progesterone-like action of Althesin was also tested (for rationale see ref. 10) in long term ovariectomised rats which had been given 20 μ g oestradiol benzoate, subcutaneously, 72 h before the administration of either 0.5 ml Althesin per 100 g, 2.5 mg progesterone or 0.5 ml arachis oil. Under ether anaesthesia, 5 h later, a blood sample was taken from the external jugular vein. The mean ($\pm$ s.e.m.) plasma LH concentrations were 18.2 ± 6.2 , 35.8 ± 13.8 and 147.5 ± 35.4 ng ml⁻¹ in animals treated with oil ($n = 6$), Althesin ($n = 6$) and progesterone ($n = 7$), respectively. There was no significant difference between the mean plasma LH concentration in the oil, compared with the Althesin-treated group; however, the concentration in the progesterone-treated animals was significantly greater than that in oil- ($P < 0.005$) or Althesin- ($P < 0.02$) treated animals.

The concentration of GnRH in pituitary stalk blood was studied in 132 animals anaesthetised at frequent intervals during the oestrous cycle. The pituitary stalk was exposed according to Worthington¹¹ and Fink and Jamieson³. Special care was taken to ensure haemostasis; in particular, damage to the tuberoinfundibular artery was avoided whenever possible. Blood was collected during 3–6 consecutive 30-min periods and plasma GnRH concentration was determined by double antibody radioimmunoassay^{3,12,13}. The lower limits of sensitivity of

these assays for 100- μ l samples (mean c.p.m. $\pm$ 2 s.d. of the total bound tubes) ranged from 6.2 to 15.8 pg GnRH ml⁻¹. These values were assigned to the relatively few samples (see Tables 1 and 2) in which the concentration of GnRH was below the limit of sensitivity of the assay. For statistical purposes, the GnRH concentrations of the 30-min collections between various times of the cycle were grouped together to determine the mean concentrations shown in Table 1. Details of the GnRH concentrations in the 30-min collections are shown in Fig. 1. There were no appreciable differences between the mean GnRH concentrations on the days of metoestrus and dioestrus, and between 0800 and 1500 of pro-oestrus. Concentrations then rose to reach a value at 1730–1800 which was significantly greater than that at any other time except between 1800 and 1830. The decline in concentration which occurred after 1830 of pro-oestrus was interrupted by a small peak between 2230 of pro-oestrus and 0200 of oestrus. There were no significant differences between the volumes of the collections between 1030 and 1830 of pro-oestrus and between 2230 of pro-oestrus and 0200 of oestrus. Table 2 shows that the GnRH concentration of pituitary stalk blood collected during Althesin anaesthesia between 1600 and 1830 of pro-oestrus was significantly greater ($P < 0.05$) than the concentration in stalk blood collected at the same time from animals anaesthetised with sodium pentobarbitone. The

Table 2 Mean ($\pm$ s.e.m.) concentrations of GnRH in pituitary stalk, 'pharyngeal' and jugular venous plasma, and volumes of collections

Source of plasma*	No. of samples	No. of samples in which GnRH detectable†	Concentration of GnRH (pg ml ⁻¹)	Blood volume (μ l per 30 min)
Stalk plasma	64	63	102 $\pm$ 23	374 $\pm$ 35
Althesin anaesthesia				
Stalk plasma	23	17	19 $\pm$ 1	459 $\pm$ 38
Sodium pentobarbital anaesthesia				
Pharyngeal plasma	31	14	25 $\pm$ 2	450 $\pm$ 61
Althesin anaesthesia				
Jugular venous plasma	40	6	19 $\pm$ 1	c.a. 500
Althesin anaesthesia				

*All samples were obtained between 1600–1830 on day of pro-oestrus.

†As for Table 1.

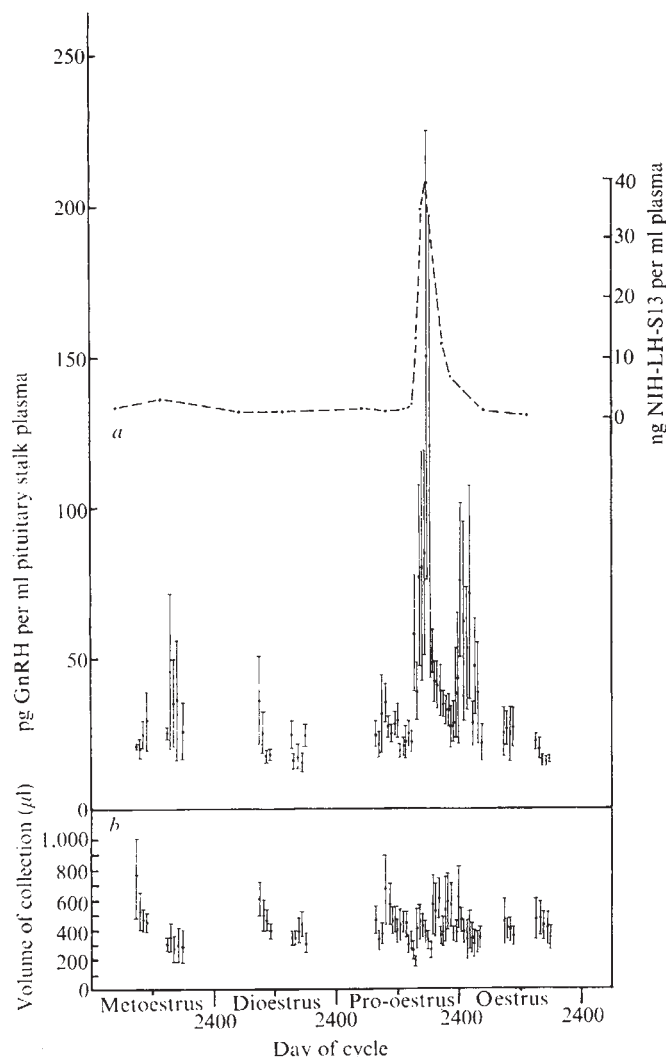


Fig. 1 Mean ($\pm$ s.e.m.) concentrations of GnRH (a) and volumes (b) of 30-min collections of pituitary stalk blood. Most means are based on 5–15 samples, a few on 3–4 samples. The mean concentrations of plasma LH (---) (data from ref. 6, with permission) are shown for comparison.

difference between the volumes of the collections was not significant. The concentration of GnRH in stalk plasma collected under Althesin was also greater ($P < 0.05$) than that in blood collected from the pharyngeal mucosa (for method see ref. 3) and in blood withdrawn from the external jugular vein ($P < 0.01$).

Comparison of the present data with peripheral plasma LH concentrations in rats of the same strain similarly maintained (Fig. 1) suggests that the LH surge begins shortly after the GnRH surge. After the peak, plasma LH concentrations fall in parallel with stalk plasma GnRH concentrations. The large variation in GnRH concentration, especially between 1600 and 1830 (values ranging from undetectable to 950 pg ml⁻¹) may be due to a pulsatile release of this hormone. Similar concentrations of GnRH in pituitary stalk blood have also been found around the mid-cycle of rhesus monkeys anaesthetised with phencyclidine hydrochloride¹⁴.

The smaller peak of stalk plasma GnRH concentration between 2230 of pro-oestrus and 0200 of oestrus may be related to the pre-ovulatory surge of plasma FSH which continues to rise after the cessation of the LH surge and reaches a peak at about 0500 of oestrus^{6,15}. This peak of GnRH concentration also occurs at about the time of maximal sexual receptivity¹⁸ and immediately precedes ovulation (0200–0400 of oestrus).

It has been established that in the rat and other spontaneous ovulators including man, there is a massive increase in the responsiveness of the anterior pituitary gland to GnRH which occurs before and during the pre-ovulatory surge of LH^{6,17}. The present results reveal the importance of this increase in pituitary response, for, even allowing for a twofold dilution of pituitary stalk collections by blood from sources other than the hypothalamic portal vessels¹, the mean concentrations of GnRH between 1600 and 1830 on the day of pro-oestrus are relatively low compared with a level of about 3,000 pg ml⁻¹ in the peripheral circulation, which is achieved 1 min after the intravenous injection of 50 ng synthetic GnRH per 100 g body weight¹³ (minimal ovulatory dose⁶). Intravenous infusion and multiple injection studies (ref. 13 and N.M.S., S.A.C. and G.F., unpublished) have shown that plasma GnRH concentrations of 100–400 pg ml⁻¹ are capable of producing a surge of LH on the afternoon of pro-oestrus provided that the pituitary is exposed to these levels for at least 30 min. Similar concentrations of plasma GnRH produce only a slight rise in plasma LH on the afternoon of dioestrus¹³. Thus, without the 50-fold increase in pituitary responsiveness to GnRH between 1330 on the day of dioestrus and 1730 on the day of pro-oestrus⁶ the spontaneous LH surge may not occur. The priming effect of GnRH¹³ which is activated by the increased secretion of GnRH during the afternoon of pro-oestrus may be responsible for ensuring that the peaks of responsiveness and the GnRH surge coincide.

We are grateful to Drs G. D. Niswender and L. E. Reichert, Jr and the NIAMDD (Bethesda), and Drs H. Gregory, J. Gormley and A. L. Walpole (ICI Pharmaceuticals) for radio-immunoassay materials. Supported by an MRC grant to G. F.

D. K. SARKAR
SHARON A. CHIAPPA
G. FINK*

Department of Human Anatomy,
University of Oxford,
South Parks Road,
Oxford OX1 3QX, UK

NANCY M. SHERWOOD

Department of Biology,
University of Victoria,
British Columbia, Canada V8W 2Y2

Received June 29; accepted October 19, 1976.

*To whom reprint requests should be addressed.

- 1 Fink, G., and Harris, G. W., *J. Physiol., Lond.*, **208**, 221–241 (1976).
- 2 Eskay, R. L., Oliver, C., Ben-Jonathan, N., and Porter, J. C., in *Hypothalamic Hormones* (edit. by Motta, M., Crosignani, P. G., and Martini, L.), 125–137 (Academic, London, 1975).
- 3 Fink, G., and Jamieson, M. G., *J. Endocr.*, **68**, 71–87 (1976).
- 4 Timms, R. J., *J. Physiol., Lond.*, **256**, 71P–72P (1976).
- 5 Niswender, G. D., Midgley, A. R., Jr, Monroe, S. E., and Reichert, L. E., Jr, *Proc. Soc. exp. Biol. Med.*, **128**, 807–811 (1968).
- 6 Aiyer, M. S., Fink, G., and Greig, F., *J. Endocr.*, **60**, 47–64 (1974).
- 7 Brown-Grant, K., Exley, D., and Naftolin, F., *J. Endocr.*, **48**, 295–296 (1970).
- 8 Greig, F., and Weisz, J., *J. Endocr.*, **57**, 235–246 (1973).
- 9 Everett, J. W., *Physiol. Rev.*, **44**, 373–431 (1964).
- 10 Brown-Grant, K., *J. Endocr.*, **62**, 319–332 (1964).
- 11 Worthington, W. C., Jr, *Nature*, **210**, 710–712 (1966).
- 12 Nett, T. M., Akbar, A. M., Niswender, G. D., Hedlund, M. T., and White, W. F., *J. clin. Endocr. Metab.*, **36**, 880–885 (1973).
- 13 Fink, G., Chiappa, S. A., and Aiyer, M. S., *J. Endocr.*, **69**, 359–372 (1976).
- 14 Feder, H. H., Brown-Grant, K., and Corker, C. S., *J. Endocr.*, **50**, 29–39 (1971).
- 15 Neill, J. D., Dailey, R. A., Tsou, R. C., Patton, J., and Tindall, G., in *Ovulation in the Human* (edit. by Crosignani, P. G., and Mishell, D. R.), 115–125 (Academic, London, 1976).
- 16 Daane, T. A., and Parlow, A. F., *Endocrinology*, **88**, 653–663 (1971).
- 17 Feder, H. H., Brown-Grant, K., and Corker, C. S., *J. Endocr.*, **50**, 29–39 (1971).
- 18 Fink, G., Aiyer, M. S., Jamieson, M. G., and Chiappa, S. A., in *Hypothalamic Hormones* (edit. by Motta, M., Crosignani, P. G., and Martini, L.), 139–160 (Academic, London, 1975).

Synthesis of luteinising hormone releasing factor and thyrotropin-releasing factor in glutamate-lesioned mice

THE arcuate nucleus (ARC) of the mammalian hypothalamus is thought to regulate tonic luteinising hormone (LH) secretion from the anterior pituitary¹. But the immunohistochemical demonstration of luteinising hormone

releasing factor (LHRF) in nerve fibres but not cell bodies of the ARC^{2,3} and the marked reduction in LHRF content after hypothalamic deafferentation⁴, suggest that the ARC is not a centre for LHRF synthesis. To clarify this issue we have used monosodium glutamate, a neurotoxin which when administered in a low dose to young animals selectively destroys ARC neurones while leaving intact axons passing through⁵⁻⁷. If the ARC perikarya synthesise LHRF, a marked reduction in the immunohistochemical staining and content of hypothalamic LHRF should be evident in glutamate-lesioned animals. Because thyroid function is unaffected by glutamate⁸, we examined brain tissue for thyrotropin releasing factor (TRF) content as a control for the LHRF studies.

Ten-day-old CD1 albino mice (Charles River) received a single injection of glutamate (subcutaneous 18 mmol per kg body weight in a 10% aqueous solution), and their litter mate controls received an equal dose of NaCl. All animals were decapitated 96 h later. By this time, orthograde degeneration and phagocytosis of tubero-infundibular axons would be expected to be complete in the median eminence (ME) and stalk median eminence (SME)⁹. Brains from seven experimental and five control animals were removed rapidly. The hypothalamus was excised and extracted in 1 ml of 2N acetic acid. The remainder of the brain was extracted in 2 ml of 2 N acetic acid. An aliquot was removed for measurement of protein concentration¹¹. An aliquot (0.3 ml) of the remainder was freeze dried, redissolved in 0.01 M phosphate-buffered saline, pH 7.4, and assayed for LHRF using a radioimmunoassay with a sensitivity of 2 pg (I.M.D.J. and S. Reichlin, in preparation). Briefly, LHRF was labelled with ¹²⁵I using chloramine T and purified on a column of Sephadex G-25. The sample, ¹²⁵I LHRF and anti-LHRF at a titre of 1:96,000 were incubated for 48 h, and bound hormone was separated from free hormone with dilute charcoal. Antibody to LHRF was raised in a rabbit immunised with synthetic LHRF conjugated to bovine thyroglobulin, by means of *bis*-diazotised benzidine, similar to that used to develop anti-TRF¹². The LHRF antiserum reacted with the LHRF decapeptide as well as with certain C-terminal fragments (nonapeptide through pentapeptide). No cross-reaction occurred with the deamidated free acid form of LHRF, TRF, somatostatin, vasopressin, thyroid or anterior pituitary hormones. Hypothalamic and extrahypothalamic TRF in the same specimens were measured by a sensitive and specific radioimmunoassay for TRF¹².

In glutamate-treated animals there was a marked reduction in the number of neuronal cell bodies within the ARC (Fig. 1). Reaction product localising immunoreactive LHRF, however, was abundant and seen throughout the rostro-caudal extent of the ME and in the upper SME, as well as in the organum vasculosum of the lamina terminalis. Similar to the distribution described in adult mammals^{2,13}, immunoreactive LHRF in glutamate-lesioned mice tended to accumulate at the tubero-infundibular sulci, and to become more intensely concentrated in caudal regions just proximal and just distal to the SME. There was no difference in staining pattern or intensity compared with

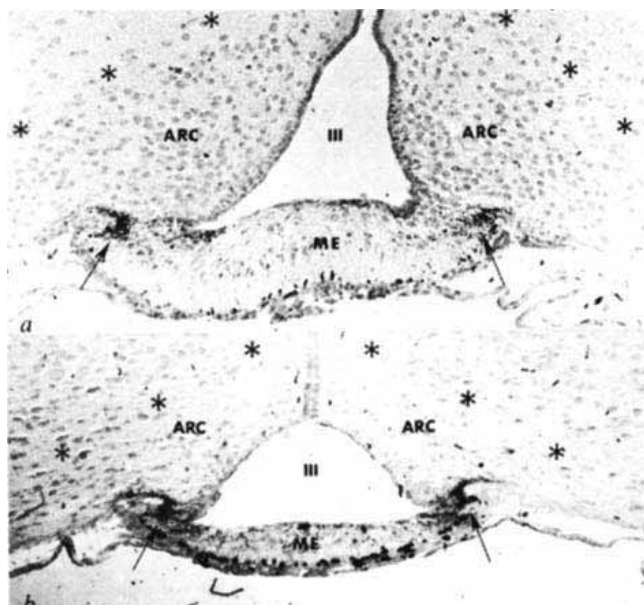


Fig. 1 Light micrograph ($\times 46$) of a frontal section through the basal hypothalamus comparing the experimental (b) with the control (a) and demonstrating intense reaction product of immunoreactive LHRF at the tubero-infundibular sulci (arrows). Note marked reduction ($> 80\%$) in the size of the arcuate nucleus (ARC). ME, median eminence; III, third ventricle.

controls. In no instance was immunoreactive LHRF seen within ARC perikarya of either NBF or Bouin's fixed tissues.

Hypothalamic LHRF content of glutamate-treated mice (mean $\pm$ s.e.m.), $1,275 \pm 106$ pg LHRF per hypothalamus ($7,734 \pm 1,389$ pg LHRF per mg protein), was similar to that seen in controls, $1,371 \pm 343$ pg per hypothalamus ($7,493 \pm 2,427$ pg LHRF per mg protein) (Table 1). In both experimental and control animals, extrahypothalamic LHRF content was very low, being at or just below the limits of sensitivity of the assay. Like LHRF, hypothalamic TRF content did not differ significantly between glutamate-treated ($2,178 \pm 165$ pg TRF per hypothalamus) and control ($2,532 \pm 159$ pg TRF per hypothalamus) animals. But TRF was readily measurable in considerable quantity in the extrahypothalamic brain of both groups (glutamate: $3,601 \pm 178$ pg TRF, control: $3,417 \pm 144$ pg TRF). These differences were not significant.

This study provides evidence that glutamate-sensitive ARC neurones do not give rise to the LHRF immunoreactive pathway, for glutamate-induced lesions, which destroyed most ARC neurones, neither altered the quantity and distribution of immunohistochemically demonstrable LHRF in the ME nor decreased total hypothalamic LHRF content. Therefore large concentrations of LHRF in the ARC, as determined by Palkovits *et al.*¹⁴, probably reflect the content of dense cored vesicles within fine calibre axons that course among ARC perikarya. Such LHRF-containing axons may also explain observations^{2,3} that immunoreactive LHRF fibres can be traced to the ARC. Their origin from

Table 1 Effect of monosodium glutamate on the hypothalamic and extrahypothalamic content of LHRF and TRF in mice (mean $\pm$ s.e.m.)

	LHRF		TRF	
	pg per tissue	pg per mg protein	pg per tissue	pg per mg protein
Hypothalamus				
Glutamate	$1,275 \pm 106$	$7,734 \pm 1,389$	$2,178 \pm 165$	$12,914 \pm 1,637$
Control	$1,371 \pm 343$	$7,493 \pm 2,427$	$2,532 \pm 159$	$13,393 \pm 2,297$
Extra-hypothalamic brain				
Glutamate	< 66	< 18.5	$3,601 \pm 178$	829 ± 68
Control	< 66	< 16.3	$3,417 \pm 144$	840 ± 57

ARC perikarya, however, need not be imposed. Furthermore, many workers have failed to confirm^{2,13,15-17} reports that mammalian ARC perikarya are immunoreactive for LHRF¹⁸⁻²⁰, and it has been suggested that perikaryal staining described in these ARC is due to a nonspecific tissue-immunoglobulin reaction^{21,17}.

The data reported here suggest that the LHRF in the ARC region of mice is located in dense cored vesicles of axons passing through the nucleus, though its presence within the small number of ARC perikarya, which are not glutamate-sensitive in the conditions of the experiment, is not excluded. Further study of the origin of the immunohistochemically demonstrable LHRF-positive fibres in the ME and upper SME is needed.

We thank Ms Judith Bollinger and Ms Faye Soohoo for assistance with radioimmunoassays. This study was supported by a USPHS training grant.

RONALD M. LECHAN

College of Medicine,
University of Vermont,
Burlington, Vermont 05401

LESLEY C. ALPERT
IVOR M. D. JACKSON*

Endocrine Division, Department of Medicine,
Tufts New England Medical Center Hospital and
Department of Anatomy, Tufts School of Medicine,
Boston, Massachusetts 02111

Received March 22; accepted October 4, 1976.

*To whom reprint requests should be sent.

- 1 Flerkó, B., in *Neuroendocrinology* (edit. by Martini, L., and Ganong, W. F.), 613-667 (Academic, New York, 1966).
- 2 Sétáló, G., Vigh, S., Schally, A. V., Arimura, A., and Flerkó, B., in *Endocrinology*, **96**, 135-142 (1975).
- 3 Sétáló, G., Vigh, S., Schally, A. V., Arimura, A., and Flerkó, B., *Brain Res.*, **103**, 597-602 (1976).
- 4 Brownstein, M. J., Arimura, A., Schally, A. V., Palkovits, M., and Kizer, J. S., *Endocrinology*, **98**, 662-665 (1976).
- 5 Olney, J. W., *J. Neuropath. exp. Neurol.*, **30**, 75-90 (1971).
- 6 Burde, R. M., Schainker, B., and Kayes, J., *Nature*, **233**, 58-60 (1971).
- 7 Paull, W. K., and Lechan, R. M., *Anat. Rec.*, **178**, 436 (1974).
- 8 Redding, T. W., Schally, A. V., Arimura, A., and Wakabayashi, I., *Neuroendocrinology*, **8**, 245-255 (1971).
- 9 Raisman, G., in *Brain-Endocrine Interaction. Median Eminence Structure and Function* (edit. by Knigge, K. M., Scott, D. E., and Weindl, A.), 109-118 (Karger, Basel, 1972).
- 10 Alpert, L. C., Brawer, J. R., Jackson, I. M. D., and Reichlin, S., *Endocrinology*, **98**, 910-921 (1976).
- 11 Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., *J. biol. Chem.*, **193**, 265-275 (1951).
- 12 Jackson, I. M. D., and Reichlin, S., *Endocrinology*, **95**, 854-862 (1974).
- 13 Baker, B. C., Dermody, W. C., and Reel, J. R., *Endocrinology*, **97**, 125-135 (1975).
- 14 Palkovits, M., Arimura, A., Brownstein, M., Schally, A. V., and Saavedra, J. M., *Endocrinology*, **95**, 554-558 (1974).
- 15 Kordon, C., Kerdellhue, B., Pattou, E., and Jutisz, M., *Proc. Soc. exp. Biol. Med.*, **147**, 122-127 (1974).
- 16 King, J. C., Parsons, J. A., Erlandsen, S. L., and Williams, T. H., *Cell Tiss. Res.*, **153**, 211-217 (1974).
- 17 Gross, D. S., *Endocrinology*, **98**, 1408-1417 (1976).
- 18 Zimmerman, E. A., Hsu, K. C., Feris, M., and Kozlowski, G. P., *Endocrinology*, **95**, 1-8 (1974).
- 19 Naik, D. V., *Cell Tiss. Res.*, **157**, 423-436 (1975).
- 20 Silverman, A. J., *Endocrinology*, **99**, 30-41 (1976).

Impairment of luteinising-hormone release following oestrogen administration to hyperprolactinaemic ewes

THE antagonistic role of prolactin during the post-partum period seems well established in many species¹⁻³. But mechanisms by which prolactin may act are not yet known. There is some evidence that prolactin may exert its inhibitory function at the ovarian level^{4,5}, but an eventual action on the hypothalamo-hypophysial axis cannot be excluded. Experiments described here have been designed to resolve this problem.

The responsiveness of the pituitary to luteinising hormone releasing hormone (LHRH) injections, was studied in eight suckling 'Préalpes du Sud' ewes. All the animals were spayed in October, 1 d after parturition, to eliminate the variable of ovarian steroid feedback. Four of the ewes were given 1 mg 2-Br- α -ergocryptine (CB 154, Sandoz) intramuscularly, twice daily from

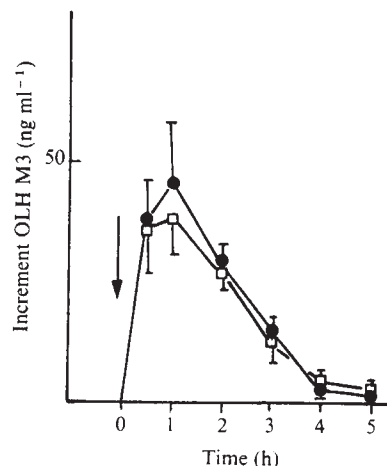


Fig. 1 LH increments in spayed ewes after intravenous injection of 200 μ g LHRH (arrow): ●, control ewes; □, TRH-treated animals. (Mean values $\pm$ standard error. Four animals in each group.)

the time of parturition to day 60 *post partum* to suppress prolactin levels⁶. The other four animals served as untreated controls with high prolactin levels. Ovine prolactin and LH were measured by radioimmunoassay^{7,8}.

Both groups were injected once a week for eight weeks with an intravenous bolus of 200 μ g synthetic LHRH (Hoechst) and an LH release was always observed in all animals. The responses were very weak on the day of parturition (before castration), but increased progressively during the first four weeks *post partum* and remained constant thereafter. Since the pattern of LH release was indistinguishable in the treated and control group, it was concluded that high prolactin levels do not modify the ability of the pituitary to discharge LH in response to LHRH.

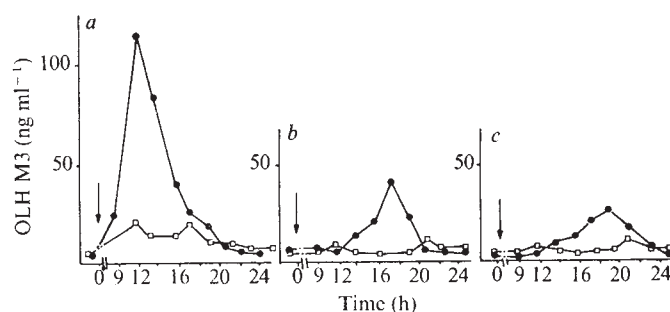


Fig. 2 Inhibition during hyperprolactinaemia of LH release attributable to oestradiol-17- β in three spayed ewes. ●, control ewes; □, TRH-treated animals. Arrows, intramuscular oestradiol-17- β : a, 25 μ g; b, 25 μ g; c, 12 μ g.

A similar experiment was performed on a group of spayed ewes that were not lactating. Intravenous LHRH (200 μ g) was given twice at 3-d intervals, on the first occasion during a control infusion with physiological saline, and the second during a time when prolactin levels had been artificially elevated by repeated infusions of thyrotropin releasing hormone (TRH), 20 μ g, 12 times a day. This produced an elevated prolactin level 48 h before the LHRH stimulation, and mimicked the suckling-induced prolactin surges⁹. Once again, the LH response to exogenous LHRH was not modified by the hyperprolactinaemia (Fig. 1). These results are also in agreement with observations on women in hyperprolactinaemic amenorrhea¹⁰⁻¹², where the LHRH response was normal. Hyperprolactinaemia in the monkey may, however, render the pituitary less responsive to LHRH¹³.

To see whether the positive feedback effect of oestradiol on the hypothalamus was impaired by high prolactin levels, ewes castrated for more than three months received at 48-h intervals three

randomised intramuscular injections of oestradiol-17- β (12, 25, 50 μ g). The most effective dose in terms of gonadotropin release differed from one animal to another. This dose of oestradiol-17- β became ineffective after the same animal had been rendered hyperprolactinaemic with TRH infusions, as described previously (Fig. 2).

These findings, together with recent data from women^{14,15}, suggest that the positive feedback effect of oestradiol on LH release^{16,17} is greatly impaired by high prolactin levels. This is probably one of the ways in which suckling inhibits ovulation.

We thank Dr R. V. Short for criticism and Mrs C. Meusnier for technical assistance. This work was supported by grants of the Délégation Générale à la Recherche Scientifique et Technique and of the Institut National de la Santé et de la Recherche Médicale.

G. KANN
J. MARTINET
A. SCHIRAR

Laboratoire de Physiologie de la Lactation
INRA, 78350-Jouy-en-Josas, France

Received August 16; accepted September 28, 1976.

¹ Tyson, J. E., Khojandi, M., Huth, J., Smith, B., and Thomas, P., *Am. J. Obstet. Gynecol.*, **121**, 375-379 (1975).

² Lu, K. H., et al., *J. Endocr.*, **68**, 241-250 (1976).

³ Kann, G., and Martinet, J., *Nature*, **257**, 63-64 (1975).

⁴ Zarate, A., Canales, E. S., Soria, J., Ruiz, F., and McGregor, C., *Am. J. Obstet. Gynecol.*, **112**, 1130-1132 (1972).

⁵ McNatty, K. P., Sawyers, R. S., and McNeilly, A. S., *Nature*, **250**, 653-655 (1974).

⁶ Kann, G., *5th Int. Congr. Endocr., Hamburg, Abstr.*, 611 (1976).

⁷ Kann, G., *C. r. hebdomadaire Séance Acad. Sci., Paris*, **272**, 2808-2811 (1971).

⁸ Kann, G., *C. r. hebdomadaire Séance Acad. Sci., Paris*, **272**, 2934-2937 (1971).

⁹ Kann, G., Habert, R., and Denamur, R., *C. r. hebdomadaire Séance Acad. Sci., Paris*, **276**, 1321-1324 (1973).

¹⁰ Thorner, M. O., McNeilly, A. S., Hagan, C., and Besser, G. H., *Brit. med. J.*, **2**, 419-422 (1974).

¹¹ Del-Pozo, et al., *J. clin. End. Met.*, **39**, 18-26 (1974).

¹² Valcke, J. C., et al., *Ann. Endocr.*, **35**, 423-443 (1974).

¹³ Maneckjee, R., Srinath, B. R., and Moudgal, N. R., *Nature*, **262**, 507-508 (1976).

¹⁴ Glass, M. R., Shaw, R. W., Logan-Edwards, R., Butt, W. R., and London, D. R., *Int. Symp. Hypoth. Endocr. Functions, Québec Abstract 21* (1975).

¹⁵ Aono, T., Miyake, A., Shioji, T., Kinugasa, T., Onishi, T., and Kurachi, K., *J. clin. End. Met.*, **42**, 696-702 (1976).

¹⁶ Yen, S. S. G., Siler, T. M., Van Den Berg, C., and Ehara, Y., *Adv. Biosci.*, **15**, 163-179 (1975).

¹⁷ Fink, G., Aiyer, M. G., Jamieson, M. G., and Chiappa, S. A., in *Hypothalamic Hormones*, (edit. by Motta, M., Crosignani, P. G., and Martini, L.), 139-160 (1975).

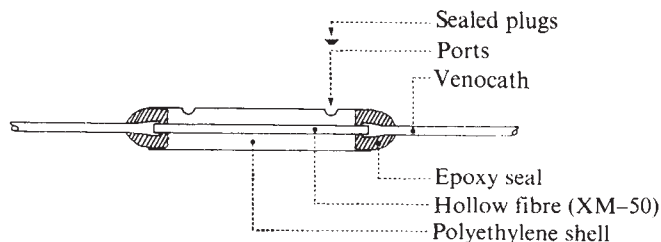


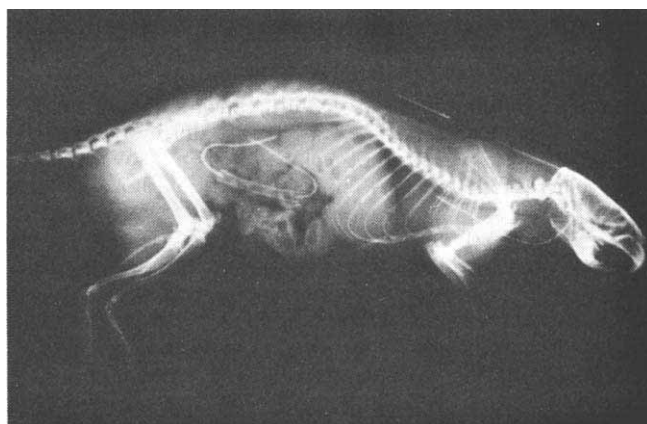
Fig. 1 Diagram to show the construction of the implantable artificial endocrine pancreas.

(310-330 g) by collagenase digestion³ and purified by Hypaque-Ficoll density gradient centrifugation⁴. Between 1,200 and 1,500 intact islets were suspended in Medium 199 supplemented with 10% foetal calf serum, penicillin (100 U ml⁻¹), streptomycin (0.1 mg ml⁻¹) and kanamycin (0.1 mg ml⁻¹) and were introduced into the artificial capillary unit through two 0.12-cm holes in the polyethylene shell. The holes were plugged with 'fitted rubber' tips (Critocap-J, Sherwood Medical Ind. Inc., St Louis) and sealed with epoxy resin.

This implantable artificial endocrine pancreas unit was perfused overnight with 125 ml of nutrient medium which was recirculated at a rate of 1.5-2.0 ml min⁻¹. Silastic tubing connecting the unit and the medium reservoir served as a gas exchanger. The circuit was operated at 37 °C in a humidified atmosphere of 5% CO₂ and 95% air.

We used male inbred Lewis rats, in which diabetes was induced by intravenous injection of streptozotocin (5.5 mg per 100 g) when they were between 300 and 320 g in body weight. An animal was defined as diabetic only when 24-h urine outputs were greater than 100 ml for at least 2 weeks and when the fasting blood glucose level was greater than 400 mg per 100 ml. Twenty-four hours before implantation, a radiopaque intravenous catheter (Venocath-16) was inserted into the jugular vein of the recipient diabetic rat to facilitate collection of blood samples, the infusion of heparin and the replacement of isogeneic blood during the subsequent experiment. The catheter was kept open with saline containing heparin (10 U ml⁻¹). The diabetic rat was fasted for 4 h before implantation which was carried out under ether anaesthesia. An incision 3 cm long was made along the linea alba and the abdominal aorta was exposed. Heparin (50 U) was injected intravenously through the femoral artery. Two clamps were applied on the abdominal aorta between the renal artery and the ilio-lumbar artery. The implant was subsequently inserted directly into the abdominal aorta.

Fig. 2 Radiogram to show the positioning of the jugular vein catheter for blood sampling and the site of the implanted artificial endocrine pancreas as a vascular shunt in the abdominal aorta.



Implantable artificial endocrine pancreas unit used to restore normoglycaemia in the diabetic rat

PARENTERAL insulin therapy has long been the primary form of treatment for patients with diabetes, but it does not prevent the chronic complications of diabetes, which involve retinopathy, neuropathy, nephropathy and vascular disease and cause the most morbidity and mortality of diabetic patients. One of the most promising alternative approaches involves transplantation of insulin-producing tissue. In animal models, sustained correction of diabetes has been accomplished by the transplantation of isolated pancreatic islets from donors syngeneic with the recipients^{1,2}. We report here experiments with an implant in which islet cells are placed outside a synthetic capillary (hollow fibre) through which smaller molecules, such as insulin, can diffuse freely. Using this device we have normalised the high glucose level of diabetic rats.

The capillary consisted of a polyvinyl chloride-acrylic copolymer (XM-50) fibre (Amicon) with a 450- μ m internal diameter which had a nominal molecular weight cutoff of 50,000. A 2.8-cm long fibre was connected by epoxy resin at both ends with 5-cm pieces of radiopaque intravenous catheter with a 0.81-mm internal diameter and 1.2-mm outside diameter (Venocath-16, Abbott). The fibre was inserted into 3 cm of polyethylene shell (Intramedic Tubing, PE320, Clay Adams, B.D. and Co., Parsippany, New Jersey) and the implantable artificial capillary unit was made by sealing the lumen of the polyethylene shell and the fibre at the fibre-catheter junction (Fig. 1).

Islets of Langerhans were isolated from male Wistar rats

Samples of 0.4 ml of blood were withdrawn before the ether anaesthetic, every hour for the first 5 h after implantation and every other hour thereafter, for blood glucose and insulin determinations (by the Beckman glucose analyser and the insulin assay kit (Amersham Searle), respectively). Withdrawn blood was replaced with isogenic blood. To maintain the circulation through the implanted unit, the recipient was administered 50 U of heparin at 2-h intervals for the first 8 h and subsequently with 100 U of heparin every 4 h.

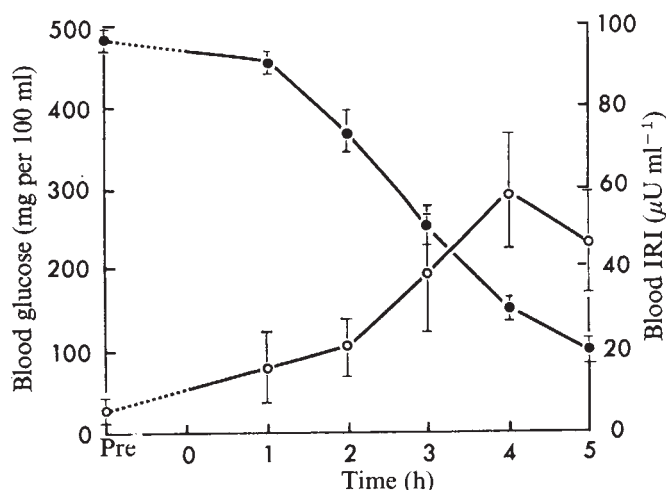


Fig. 3 Decline in blood glucose levels in diabetic rats after the implantation of an artificial endocrine pancreas. Pre indicates the sample taken before ether anaesthesia for the implantation procedure. Time 0 represents the connection of the artificial endocrine pancreas as a shunt in the circulatory system. The values expressed are the $X \pm s.e.$ blood glucose and insulin levels of eight diabetic rats receiving such implants.

The positioning of the jugular vein catheter and the implanted artificial endocrine pancreas are shown radiologically in Fig. 2. In all the recipient streptozotocin-induced diabetic rats blood sugar levels started to decline within 2 h and achieved normoglycaemia between 4 and 5 h after implantation. There was a corresponding increase in blood insulin levels.

Figure 3 shows the trend of changes in blood glucose and circulating insulin in eight diabetic rats receiving

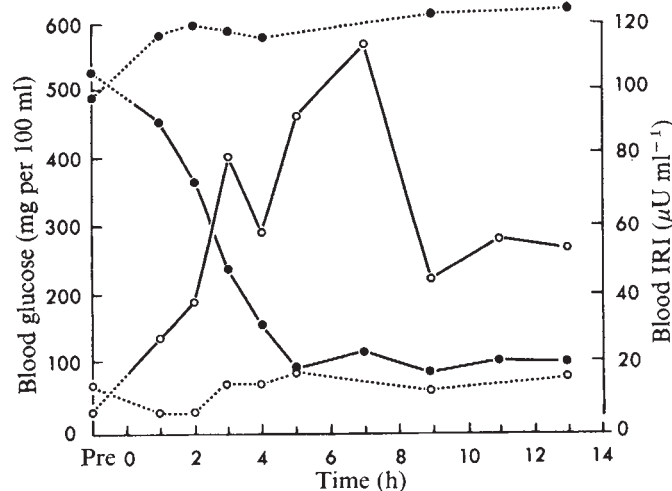


Fig. 4 The blood glucose and insulin levels in diabetic rats following the implantation of an artificial endocrine pancreas (—), and in diabetic rats following the implantation of an artificial capillary unit without islets (---). Each point represents the average value for two animals.

implants. The limited duration of study in this group was a consequence of excessive bleeding due to the effect of heparinisation. Figure 4 shows changes in insulin and blood sugar in two recipients which survived longer after implantation. Two diabetic control rats, given a capillary unit without islets, showed persistent hyperglycaemia and low insulin levels. There was clearly an improvement of hyperglycaemia and increased circulating insulin levels in diabetic rats after implantation with functional islets inside the artificial capillary unit.

We have shown already that the functional capacity of intact islets cultured in artificial capillary culture units *in vitro* is maintained for at least 97 d (ref. 5). Cultured islets responded to increased glucose concentration by increased insulin production throughout the culture period and the maintenance of the functional capacity of islets within the artificial capillary unit *in vivo* may also be anticipated. Although the *in vivo* studies were of limited duration, our results show that the implanted unit is sensitive to glucose and can produce sufficient insulin to normalise the high blood sugar levels in the diabetic rats within 4 h of implantation.

The potential importance of this approach is to provide functional pancreatic islets in the diabetic recipient which can supply insulin, glucagon and possibly other islet factors, in response to physiological needs and ultimately ameliorate insulin-requiring diabetes and perhaps prevent the development of complications. Islets within the unit may be protected from immune destruction by circulating host lymphocytes and antibodies, by virtue of the selective permeability of the molecular size of the synthetic capillaries. Thus, the artificial endocrine pancreas unit would mimic an immunologically privileged site. The system, if successful in the allogeneic rat, would have clinical potential.

This work was supported by grants from the MRC of Canada, the British Columbia Medical Research Foundation and the Charles Best Fund of the Canadian Diabetic Association. We thank Amicon Corporation for fibres, and Dr K. A. Evelyn for support and advice.

WAH JUN TZE
FOOK CHUEN WONG
LYDIA M. CHEN
SIMON O'YOUNG

G. F. Strong Laboratory for Medical Research,
Department of Paediatrics,
University of British Columbia,
Vancouver, British Columbia V5X 1X2, Canada

Received July 26; accepted September 30, 1976.

- 1 Ballinger, W. C., and Lacy, P. E., *Surgery*, 72, 175-186 (1972).
- 2 Leonard, R. J., Lazarow, A., and Hegre, O. D., *Diabetes*, 22, 413-428 (1973).
- 3 Lacy, P. E., and Kostianovsky, M., *Diabetes*, 16, 35-39 (1967).
- 4 Tze, W. J., Wong, F. C., and Tingle, A. J., *Transplantation*, 22, 201-205 (1976).
- 5 Tze, W. J., and Chen, L. M., *Diabetes* (in the press).

Carbocyanine dyes inhibit Ca-dependent K efflux from human red cell ghosts

CARBOCYANINE dyes are fluorescent probes of membrane potential in red cells^{1,2}, and their application has been extended to Ehrlich ascites cells^{3,4} and phytoplankton⁵. I have used them to study the effect of potassium concentration gradients on the membrane potential of resealed ghosts containing Ca buffers. Intracellular Ca specifically renders these ghosts highly permeable to K (ref. 6). During this work, I discovered that 3,3'-dipropyl- and 3,3'-diethylthiadicarbocyanine iodide cause a progressive inhibition of K efflux at low external K concentrations, and that external K protects against the inhibitory effects of the dyes. The results suggest that the carbocyanine dyes are specific inhibitors of the Ca-dependent K transport system.

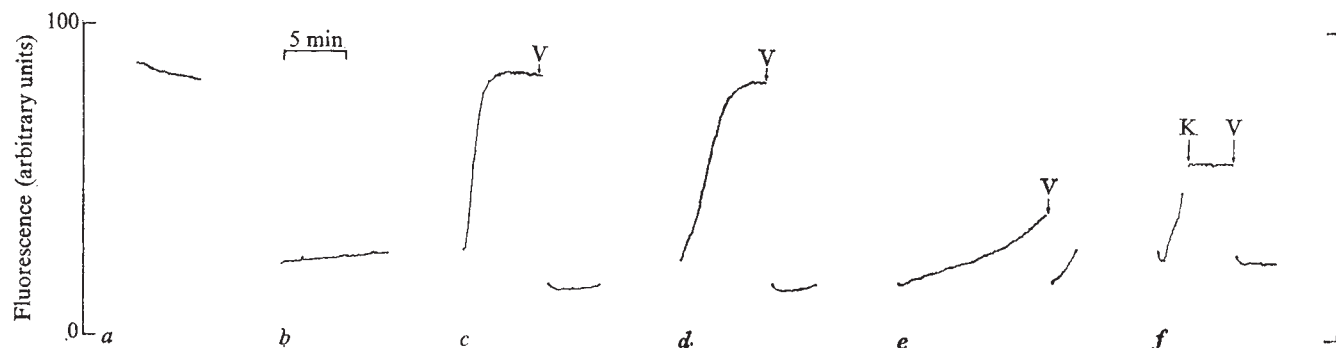


Fig. 1 Recordings of ghost suspensions dyed with 280 nM 3,3'-diethylthiadicarbocyanine iodide. The ghosts were prepared to contain 2 mM Ca, 3 mM HEDTA, 2 mM phosphate and about 100 mM KCl (ref. 6), and were washed in a 100-mM K solution. The solutions used contained 100 mM (K+choline) 98 mM Cl, 3 mM Tris, 2 mM phosphate and 0.2 mM EGTA, and had a pH of 7.1 at 37 °C. In each record, 20 μ l of concentrated dye (in ethanol) was added to 3 ml of solution in a cuvette; 10 μ l of packed ghosts was added, mixed by inversion (Parafilm), and recording started about 10 s later. A Perkin-Elmer 204 fluorimeter was used with excitation wavelength 620 nm and analysing wavelength 660 nm; the temperature was 37 °C, and the amplification kept unchanged throughout. Before addition of the ghosts, the K concentration in the cuvette was *a*, 100 mM; *b*, 2 mM; *c*, 0 mM; *d*, *f*, 0.1 mM; *e*, 0.5 mM. Addition of the ghosts increased external K by about 0.1 mM in *b*–*f* because of K in the extracellular fluid and the presence of unsealed ghosts. A sample (10 μ l) of 0.3 mM valinomycin solution (in ethanol) was added to the cuvette at the arrows marked V, and 60 μ l of 100 mM KCl solution at the arrow marked K. A large fluorescence signal (as in *a*) corresponds to a small membrane potential, and a small signal (as in *b*) corresponds to a large hyperpolarisation. Membrane potential is related monotonically to fluorescent intensity^{1,2,8}. Qualitatively identical results were obtained with 3,3'-dipropylthiadicarbocyanine iodide.

Figure 1 shows recordings of fluorescence from 0.33% ghost suspensions dyed with 280 nM 3,3'-diethylthiadicarbocyanine iodide. The ghosts contained 100 mM K and 5 μ M free Ca; the external K and choline concentrations were varied, keeping their sum 100 mM. Figure 1*a* shows a large fluorescence signal from a suspension of ghosts in a 100-mM K medium, and *b* shows the small signal seen in a 2-mM K medium. This corresponds to a large hyperpolarisation in *b*, caused by the outward K gradient and the high K permeability of the ghosts^{2,6}. In record *c* the ghosts were suspended in a K-free medium. The initial

response was a large hyperpolarisation, followed by a rapid depolarisation to zero potential. Such a depolarisation might be caused by an equalisation of internal and external K, an increase in Cl permeability or a decrease in K permeability. Subsequent addition of 1 μ M valinomycin (arrow V) caused a large hyperpolarisation, indicating that a large K gradient was still present. The (dipropyl) dye greatly reduces the rate of loss of K from the ghosts (Fig. 2), so the depolarisation seen in Fig. 1*c* is caused by a decrease in K permeability. Figure 1*d* and *e* shows that a similar response is seen in solutions containing 0.1 mM K and 0.5 mM K, but the rate of depolarisation is reduced as the K concentration is raised. In Fig. 1*f* the ghosts were suspended in a 0.1-mM K solution, but the K concentration was increased by 2 mM during the depolarising phase (arrow K). This prevented further depolarisation, and the membrane potential was held at an intermediate value. Subsequent addition of valinomycin again produced a large hyperpolarisation. Similar experiments (not illustrated) showed that Rb and Cs ions are also able to prevent the depolarisation seen in low K solutions, whereas Na, Li, choline and tetraethylammonium ions are ineffective. This is the same cation selectivity as that of the Ca-dependent K transport system⁷. It seems likely that the depolarisations seen in Fig. 1 reflect a specific association between the dye and the K transport system, and the antagonistic effect of external K suggests that the reaction occurs at the external surface of the membrane. The specific nature of the interaction is confirmed by the lack of effect of the

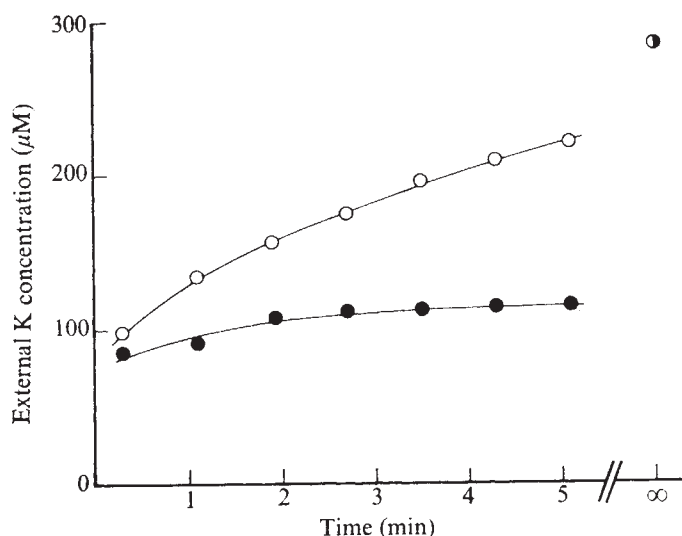


Fig. 2 Inhibition of net K efflux by 360 nM, 3,3'-dipropylthiadicarbocyanine iodide. Packed ghosts (30 μ l) (as in Fig. 1) were added to 9 ml of 0-K (choline) medium at 37 °C (as in Fig. 1), either with (●) or without (○) 60 μ l of concentrated dye solution in ethanol. Samples of suspension (1 ml) were taken at intervals, and centrifuged at 8,000 *g* for 20 s (Eppendorf 3200); timing was from the suspension of the ghosts to the start of the centrifuge. The K concentration in the supernatant was measured by flame photometry, and the points give the mean of two trials. The point at infinite time gives the average concentration of K in the whole suspension. The high initial K concentration arises for the same reasons as in Fig. 1.

Table 1 Lack of effect of 3,3'-diethylthiadicarbocyanine iodide on pump K influx

	Ouabain –	Ouabain +
Dye –	0.94 $\pm$ 0.05	0.06 $\pm$ 0.02
Dye +	0.90 $\pm$ 0.03	0.04 $\pm$ 0.01

Washed human red cells (blood bank) were suspended at 37 °C, haematocrit 0.7%, in a solution containing 150 mM choline Cl, 8 mM Tris Cl, 2 mM Tris phosphate, 5 mM glucose and 0.16 mM ⁴²KCl (pH 7.3). When present, ouabain was at 70 μ M and the dye at 300 nM. For each condition, samples were taken in triplicate at 0, 20, 40 and 60 min, centrifuged, and the cells washed three times in ice-cold incubation medium lacking ⁴²K. The influxes (in mmol per 1 cells h⁻¹, $\pm$ s.d.) were calculated from plots of the radioactivity in the cells against time.

dye on the Na pump-catalysed influx of K into red cells suspended in a 0.16-mM K medium (Table 1).

The experiments illustrated in Figs 1 and 2 monitor a changing situation during the onset of inhibition. Not only is the degree of inhibition increasing, but the internal and external dye concentrations are changing during this period⁴. In spite of this, a rough dose-response curve (Fig. 3), constructed from a series of observations of K efflux rates, shows 50% inhibition of K efflux by about 20–50 nM 3,3'-diethylthiadicarbocyanine iodide at 0.1 mM external K. This is likely to be an overestimate of the half-inhibition constant because the measurements were made during the onset of inhibition, rather than at equilibrium.

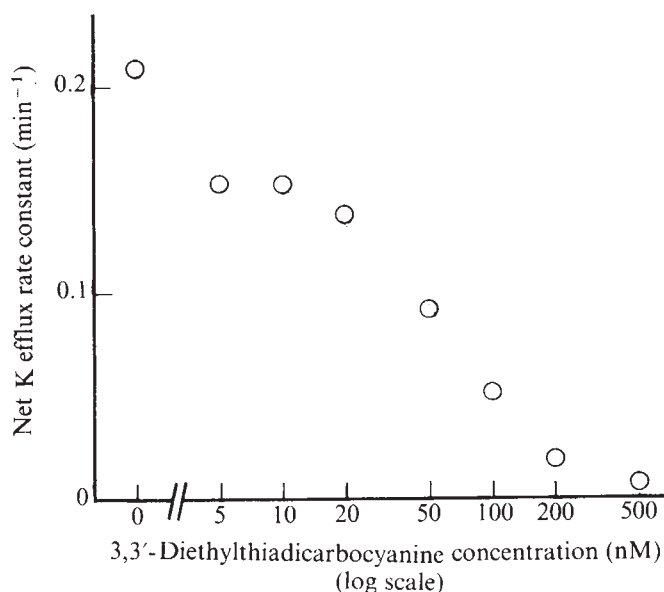


Fig. 3 Dose-response curve for the inhibition of net K efflux by 3,3'-diethylthiadicarbocyanine iodide. Each point gives the weighted mean of two determinations, from measurements made as in Fig. 2. To make a quantitative estimate of the rate of K efflux it was assumed (incorrectly) that external K would approach its equilibrium value exponentially, and the rate constants obtained from the slopes of straight lines fitted to plots of log (fraction of K remaining in ghosts) against time.

Preliminary experiments have also shown that, at fixed dye concentrations of 400 nM, 3,3'-diethylthiadicarbocyanine iodide and 3,3'-diethylthiatricarbocyanine iodide are as effective as 3,3'-diethyl- and 3,3'-dipropylthiadicarbocyanine iodide in inhibiting net K efflux from Ca-containing ghosts, whereas 3,3'-diethyloxacarbocyanine iodide and 3,3'-diethyl-9-ethyloxacarbocyanine iodide are less effective, and 3,3'-diethylthiacyanine iodide, 1,1'-diethyl-2,2'-pyridylcarbocyanine iodide and 1,1'-diethyl-2,2'-carbocyanine iodide are only slightly effective or ineffective. 3,3'-Diethylthiadicarbocyanine iodide (250 nM) also inhibits the net K loss from intact human red cells treated with 0.1 mM Pb acetate⁹, or loaded with Ca by 2 h preincubation with inosine + iodoacetamide + CaCl₂ (refs 10, 11).

Two important conclusions should be drawn from these findings. First, these carbocyanine dyes should not be assumed to be inert probes of membrane potential, even at nanomolar concentrations. Second, and perhaps more positively, some carbocyanine dyes might prove very useful in the study of Ca-dependent K permeability systems, which seem to be widely distributed in nature^{12–14}. For example, they might be used to dissect ionic currents in voltage-clamp studies, or even as a marker during membrane purification.

I thank Dr B. Salzberg for a gift of 3,3'-dipropylthiadicarbocyanine iodide, the Director of the South London Transfusion Centre for blood, the Wellcome Trust for equipment, and Professor P. F. Baker for helpful discussions.

T. J. B. SIMONS

Department of Physiology,
University of London King's College,
Strand, London WC2R 2LS, UK

Received August 5; accepted October 4, 1976.

- 1 Sims, P. J., Waggoner, A. S., Wang, C.-H., and Hoffman, J. F., *Biochemistry*, **13**, 3315–3330 (1974).
- 2 Hoffman, J. F., and Laris, P. C., *J. Physiol., Lond.*, **239**, 519–552 (1974).
- 3 Philo, R. D., and Eddy, A. A., *Biochem. Soc. Trans.*, **3**, 904–906 (1975).
- 4 Laris, P. C., Pershadsingh, H. A., and Johnstone, R. M., *Biochim. biophys. Acta*, **436**, 475–488 (1976).
- 5 Adamich, M., Laris, P. C., and Sweeney, B. M., *Nature*, **261**, 583–585 (1976).
- 6 Simons, T. J. B., *J. Physiol., Lond.*, **256**, 209–225 (1976).
- 7 Simons, T. J. B., *J. Physiol., Lond.*, **256**, 227–244 (1976).
- 8 Hladky, S. B., and Rink, T. J., *J. Physiol., Lond.*, **258**, 100P–101P (1976).
- 9 Riordan, J. R., and Passow, H., *Biochim. biophys. Acta*, **249**, 601–605 (1971).
- 10 Gardos, G., *Acta physiol. hung.*, **10**, 185–189 (1956).
- 11 Lew, V. L., *Biochim. biophys. Acta*, **233**, 827–830 (1971).
- 12 Meech, R. W., *J. Physiol., Lond.*, **237**, 259–277 (1974).
- 13 Krnjević, K., and Lisiewicz, A., *J. Physiol., Lond.*, **255**, 363–390 (1972).
- 14 Barrett, E. F., and Barrett, J. N., *J. Physiol., Lond.*, **255**, 737–774 (1976).

Intercellular junctions of frog skin epithelial cells

It has been suggested that junctional complexes between epithelial cells provide intercellular exchange of electrolytes^{1–3}, which will result in more or less coupled behaviour and functional homogeneity of neighbouring cells. In epithelia, such as frog skin, built up of several layers, electrolyte exchange through low-resistance junctions between the different cell layers would, in addition, allow participation of all layers in active transepithelial transport of ions and increase the amount of ATPase involved in transport. Na exchange between the different layers of the frog skin epithelium has recently been demonstrated in K⁺-depolarised Ouabain-poisoned skins⁴. The microelectrode experiments reported here show that low resistance connections between the different cell layers exist even under normal conditions.

Abdominal skins of *R. temporaria* and *R. esculenta* were punctured from the epithelial side perpendicular to the surface. The epithelium was properly attached to a copper grid at the corial side by a negative pressure of ~ 30 cm H₂O. Trans-epithelial potential difference (PD_{tr}) and short circuit current (I_{sc}) were measured using an automatic device which changed rhythmically between open circuit (o.c.) and short circuit (s.c.). During the o.c. periods, current pulses (5 μ A, 0.5 s) were applied transepithelially. The instantaneous change in PD_{tr} served to calculate the skin resistance ($R_t = \Delta PD_{tr}/I$). The microelectrodes (3 M KCl, $R = 4–8$ M Ω , tip potential < 5 mV) were moved by a 1- μ m step motor drive (Narishige). To ensure that any structure once penetrated by the microelectrode could not be penetrated again, the microelectrode was moved only in the forward direction during the individual observation periods. The microelectrode recorded:

PD_{sc} = potential under s.c. conditions; PD_0 and PD_b = potential difference between respectively epithelial and corial side, and microelectrode under o.c. conditions ($PD_b = PD_0 + PD_{tr}$); R_0 = resistance between epithelial side and microelectrode calculated from the instantaneous change of PD_0 upon trans-epithelial injection of current. In a part of the experiments, R_0 was estimated from $(PD_{sc} - PD_0)/I_{sc}$ and R_t from PD_{tr}/I_{sc} . All electrical parameters were registered on a two-channel chart recorder. The epithelial bathing solution (Cl or SO₄ Ringer) could be exchanged in about 1 s. Na-free solution was prepared by substituting K for Na.

Impalement of two successive cell layers occurred in eight experiments by chance. Figure 1 shows the redrawn record of one observation. After penetration of dense layers, the microelectrode recorded: $PD_{sc} = -54$ mV, $PD_0 = -1$ mV, $PD_b = -99$ mV and had passed 75% of R_t . Na-free epithelial perfusion produced a reversible change of PD_{sc} to -88 mV and PD_0 to -44 mV, while PD_b remained essentially unchanged. Changes of R_t and R_0 are quantitatively the same. According to recent investigations^{5,6} these data are typical for intracellular location of the microelectrode. Microelectrode advancement resulted in a spontaneous breakdown of the potentials to values close to those expected for the corial solution. Most likely, the microelectrode had entered an intercellular space between the epithelial cells. Part of the transepithelial resistance, however, was still in front of the microelectrode, suggesting a contribution of the intercellular spaces to R_t . The microelectrode entered an intracellular space again at 43 μ m. Potentials, resistance and response to Na-free epithelial perfusion were similar to those from the first cellular impalement. The view that a cell was punctured beneath that impalement at first and lost thereafter is supported by the fact that, in several hundred impalements of the frog skin epithelium, no cell could be repunctured. After spontaneous breakdown, a third jump to potentials of the same range as in the more outward cellular layers was observed at 52 μ m. They were stable for 20 s only, but might indicate impalement of a third cell layer before the microelectrode had passed the epithelium.

Amiloride (10^{-4} M) was used in a second impalement of the same skin to block Na entrance. The response of PD_{sc} and I_{sc} occurred without measurable delay (0.2 s) in the first and the second cell layer, also supporting the idea of electrical coupling, since alterations of the intracellular ion content would require periods of several seconds. Table 1 summarises the values of PD_{sc} , PD_b and R_0/R_t . No systematic difference exists between the two cell layers, suggesting good connection of their intracellular spaces. Although the resistance of the intercellular

junctions cannot be computed from these values (this would require impalement of several cells at given distances as performed in other epithelia^{1,3,7,8}), the order of magnitude can be estimated from a comparison of the resistances presented by the apical membrane (R_a) of the two successive layers. R_a can be calculated from

$$\frac{1}{R_t} = \frac{1}{R_s} + \frac{1}{R_a + R_b}$$

if R_s , the paracellular shunt resistance, is assumed to be measured by R_t after Amiloride (10^{-4} M) and the voltage divider ratio $PD_0/PD_b = R_a/R_b$ is used^{7,8}. The following values were obtained from the experiment, presented in Fig. 1 ($R_s = 6.8$ k Ω cm²): $R_a = 2.8$ k Ω cm² (control) and 8.0 k Ω cm² (Na free) in the first cell layer; $R_a = 3.0$ k Ω cm² (control) and 7.9 k Ω cm² (Na free) in the second layer. Appreciation of possible errors excludes the possibility that the difference in R_a , that is the resistance between the two layers, exceeds 0.3 k Ω cm². The resistance of the basolateral membranes (R_b) was considerably higher (0.9 k Ω cm² in control and 1.2 k Ω cm² with Na-free epithelial perfusion). Ion exchange between the different layers is thus less impeded than membrane passage and will provide distribution of Na through all cell layers. This must not exhibit characteristics of a single compartment since entrance across the outer border will be followed by fast distribution of Na within the cytoplasm of the first reacting cell layer, while passage into deeper layers requires penetration through—at least—space restricting junctional complexes. In addition, a certain fraction of the Na that has entered is already extruded into the intercellular spaces. This may explain why only one component during positive or negative current transients could be detected by Morel and Leblanc⁴ in transporting frog skins. But if Ouabain blocked Na extrusion

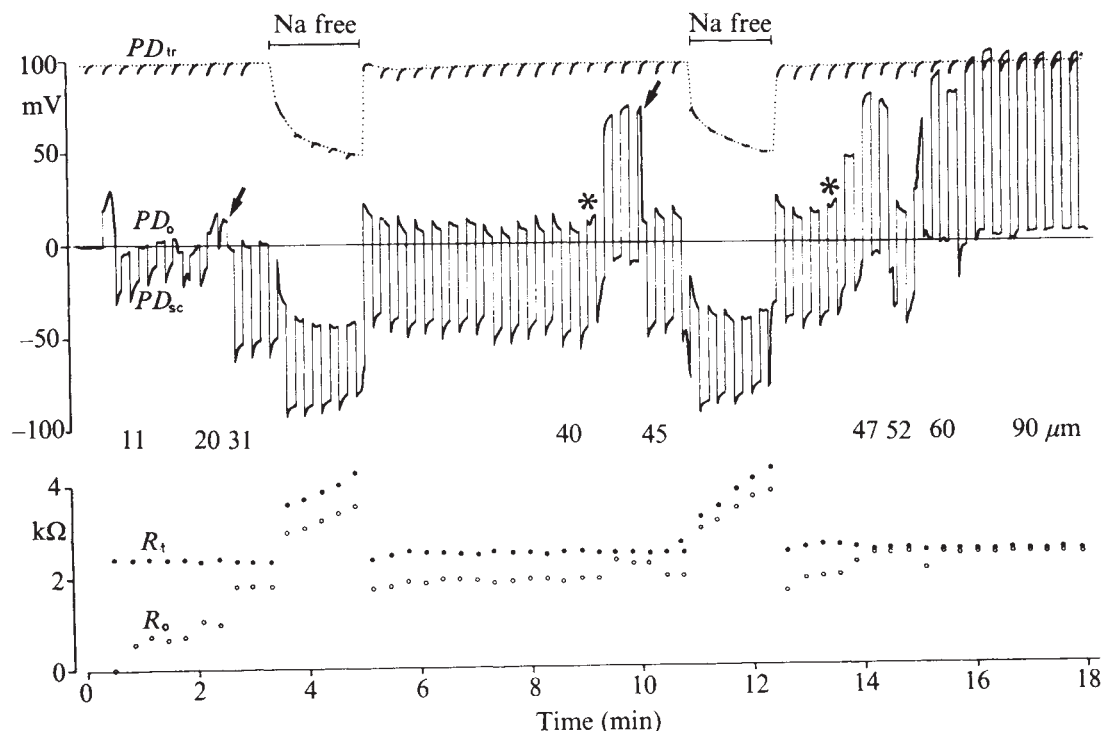


Fig. 1 Microelectrode impalement of the skin of *R. esculenta* (SO_4 -Ringer) showing recording from two successive cell layers with intermediate location of the microelectrode in extracellular (corium facing) spaces. Access to intracellular spaces is labelled by solid arrows; indications of cell rupture (breakdown) are marked by asterisks. Potentials given are with respect to the epithelial bathing solution. Trans-epithelial potential (PD_{tr}) and potential difference across the outer border were recorded at the same time; PD_{sc} was recorded during short-circuiting the skin (automatically every 6 s for 6 s). The microelectrode position is given in μ m relative to the skin surface. R_t and R_0 are calculated from the instantaneous changes of PD_{tr} and PD_0 upon transepithelial injection (5 μ A). The skin surface was indented by the microelectrode. Thus, advancement would not be transferred into distance penetrated within the epithelium, but indicates maximal values of microelectrode movement.

Table 1 Mean values of PD_{sc}^* , $PD_b^\dagger$ and $R_o/R_i^\ddagger$

a PD_{sc} (mV)	b cell 2 cell 1	a PD_b (mV)	b cell 2 cell 1	a R_o/R_i	b cell 2 cell 1
-64 ± 7	1.031 ± 0.060	-97 ± 6	0.980 ± 0.031	0.758 ± 0.035	1.060 ± 0.022

*Short-circuit intracellular potential.

†Potential difference across the basolateral membranes.

‡Fraction of the transepithelial resistance penetrated by the microelectrode.

Values observed in the first impaled cell layer are in columns *a*; columns *b* contain the ratios of these values to those recorded after intermediate extracellular location from the second cell layer. $n = 8$; $\pm$ s.e.m.

larger amounts of Na exchanged across the junctional complexes and became evident as a second component.

These microelectrode observations which demonstrate that intercellular exchange of ions occurs in normal (unpoisoned and non-depolarised) frog skins are in accordance with previous suggestions from histochemical ATPase localisation⁹. The outermost reacting cell layer¹⁰ would then be the dominating location of active Na extrusion into the intercellular spaces but at least at high rates of influx, excess Na may pass into deeper cell layers to be transported out of this facultative transport pool.

This work was supported by the Deutsche Forschungsgemeinschaft.

WOLFRAM NAGEL

Physiologisches Institut der Universität,
Pettenkoferstrasse 12,
D-8000 München 2, West Germany

Received July 28; accepted October 12, 1976.

- Loewenstein, W. R., and Kanno, Y., *J. Cell Biol.*, **22**, 565–586 (1964).
- Farquhar, M. G., and Palade, G. E., *J. Cell Biol.*, **26**, 263–291 (1965).
- Loewenstein, W. R., *Ann. N.Y. Acad. Sci.*, **137**, 441–472 (1966).
- Morel, F., and Leblanc, G., *Pflügers Arch. ges. Physiol.*, **358**, 135–157 (1975).
- Nagel, W., *Pflügers Arch. ges. Physiol.*, **365**, 135–143 (1976).
- Helman, S. I., and Fisher, R. S., *Fedn Proc.*, **35**, 702 (1976).
- Frömter, E., *J. Membr. Biol.*, **8**, 259–301 (1972).
- Reuss, L. E., and Finn, A. L., *J. gen. Physiol.*, **64**, 1–25 (1974).
- Farquhar, M. G., and Palade, G. E., *J. Cell Biol.*, **30**, 359–379 (1966).
- Voute, C. L., and Hanni, S., in *Alfred Benson Symposium: Transport Mechanism in Epithelia*, 86–98 (Munksgaard, Copenhagen, 1973).

Incorporation of $^{32}\text{P}_i$ into pyruvate dehydrogenase phosphate in mitochondria from control and insulin-treated adipose tissue

PYRUVATE dehydrogenase complexes (EC 1.2.4.1) isolated from mammalian sources have been shown to be inactivated by phosphorylation of the α subunit of the pyruvate decarboxylase component. This covalent modification is brought about by a tightly bound, ATP-requiring kinase, which is activated by NADH and acetyl CoA and inhibited by pyruvate, Ca^{2+} , thiamine pyrophosphate and ADP. Reactivation is catalysed by a specific phosphatase which requires both Mg^{2+} and Ca^{2+} (refs 1–6). In rat epididymal fat pads, the proportion of the complex in the active non-phosphorylated form is increased following brief exposure of pads to insulin^{6–8} and this effect persists during preparation and subsequent incubation of mitochondria with oxidisable substrates such as oxoglutarate and malate^{9,10}. The mechanism by which the interaction of insulin with the cell membrane results in the changes of this mitochondrial enzyme system has not been established. It is possible that insulin may act through activation of the phosphatase by an increase in mitochondrial Ca^{2+} concentration and this possibility has been explored extensively in this laboratory^{3,9,11}. In contrast, other workers^{7,8} have argued that the effect of insulin is brought about by an inhibition of the

kinase caused by a lowering of the mitochondrial concentration ratio ATP:ADP. We report here evidence against the latter hypothesis.

In fat pad mitochondria incubated with oxoglutarate and malate in the presence of $^{32}\text{P}_i$, we have found that the only appreciably phosphorylated protein component within the mitochondria is the α subunit of pyruvate dehydrogenase. Moreover, since the incorporation of phosphate into this subunit is slow compared with the equilibration of mitochondrial ATP with the medium $^{32}\text{P}_i$, the rate of incorporation gives an estimate of the kinase activity within the intact mitochondria. No evidence was found for any decrease in kinase activity in fat pad mitochondria from insulin-treated tissue—indeed, its activity seemed to be increased.

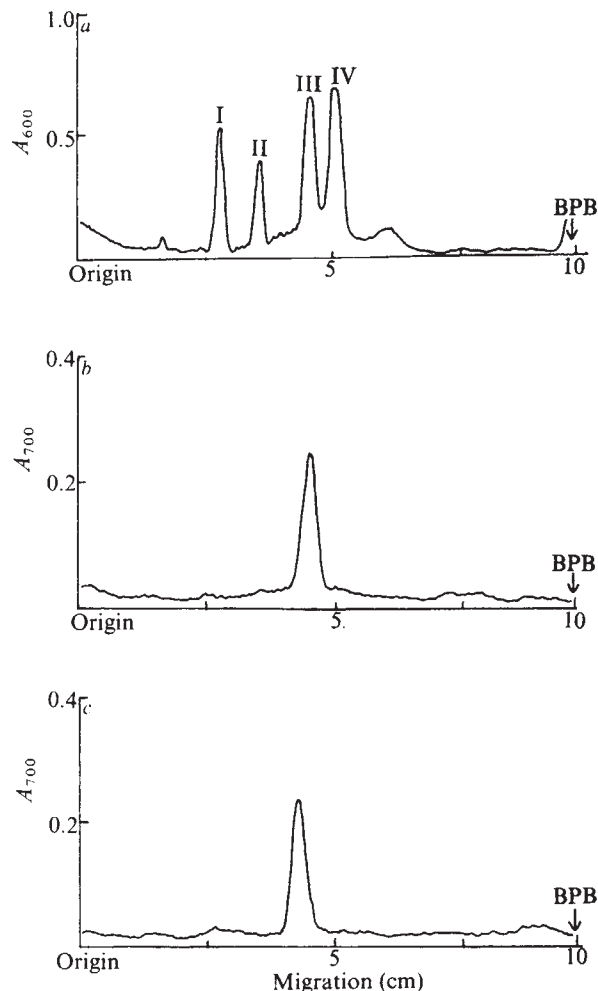


Fig. 1 SDS-polyacrylamide gel electrophoresis of components of *a, b*, pig heart ^{32}P -pyruvate dehydrogenase phosphate and *c*, of a perchloric acid precipitate of fat pad mitochondria following incubation with $^{32}\text{P}_i$ (0.2 mM and 3,000 c.p.m. μmol^{-1}) for 10 min. Samples were suspended in 50 μl of Bromophenol blue (BPB; 0.2 mg ml^{-1}), sodium dodecyl sulphate (SDS; 40 mg ml^{-1}), sucrose (0.2 g ml^{-1}), sodium phosphate (0.02 M), pH 7.0, and then incubated for 1.5 h at 60 °C. A portion (10 μl) was applied to a 7.5% (w/v) polyacrylamide gel (0.6 cm $\times$ 10 cm) containing bisacrylamide (2.7 mg ml^{-1}), SDS (1 mg ml^{-1}) and 50 mM sodium phosphate, pH 7.0 (ref. 12). Electrophoresis was carried out at 20–25 °C for about 3 h at 8 mA per tube. After electrophoresis protein components were stained with Coomassie brilliant blue and the gels scanned at 600 nm (*a*). I, Dihydrolipoate transacetylase; II, dihydrolipoate dehydrogenase; III, pyruvate decarboxylase, α subunit; IV, pyruvate decarboxylase, β subunit². The ^{32}P -labelled components were located by autoradiography after the gels were washed in 7% acetic acid overnight, and the autoradiographs scanned at 700 nm (*b* and *c*). (For preparation of ^{32}P -pyruvate dehydrogenase phosphate, see ref. 3.)

Epididymal fat pads were obtained from fed male Wistar rats (180–220 g), incubated for 30 min in bicarbonate-buffered medium containing fructose (2 mg ml⁻¹) with or without insulin (10 munit ml⁻¹), and mitochondria prepared as previously described⁹. Mitochondria (about 2 mg protein ml⁻¹) were pre-incubated in air-equilibrated KCl (125 mM), EGTA (2 mM), Tris-HCl (25 mM) pH 7.4 containing 2-oxoglutarate (2 mM), malate (0.2 mM) and potassium phosphate (0.2 mM) at 30 °C for 3 min. As found previously¹⁰, the pyruvate dehydrogenase activity had by this time reached a steady-state value which remained constant for the next 10–15 min. In mitochondria from control tissue about 15% of the complex was in the active form and this was increased in mitochondria from insulin-treated tissue to about 25%. Addition of ³²P_i was made after the 3-min preincubation and incubation continued at 30 °C for varying times up to 20 min. For measurement of ³²P_i incorporation into the α subunit of pyruvate dehydrogenase, incubations were terminated by addition of perchloric acid (final concentration 2%). Insoluble proteins were precipitated by centrifugation (90 s at 10,000g in Eppendorf 3200 minifuge) and analysed by SDS–polyacrylamide electrophoresis as described in legends to Figs 1 and 2. Recovery of ³²P-labelled pig heart pyruvate dehydrogenase phosphate by this procedure was greater than 90%. For the measurement of ATP specific activity, mitochondria were separated from the ³²P_i-containing medium by centrifugation through an oil layer into a perchloric acid–glycerol mixture (see legend to Fig. 2 for details). Measurements of initial pyruvate dehydrogenase, total pyruvate dehydrogenase and glutamate dehydrogenase activities were made as described previously^{9,11} in samples of mitochondria incubated in identical conditions but without addition of ³²P-phosphate. For pyruvate dehydrogenase phosphate, a unit of enzyme activity is that amount which yields one unit of pyruvate dehydrogenase ($\mu\text{mol min}^{-1}$ at 30 °C) after complete conversion into the non-phosphorylated form with phosphatase in the presence of Mg²⁺ and Ca²⁺.

As expected, SDS–polyacrylamide electrophoresis of the proteins in fat pad mitochondria indicated the presence of a large number of different components. But after incubation of the mitochondria with ³²P_i in the presence of oxoglutarate and malate, only a single, symmetrical peak of radioactivity was separated by electrophoresis (Fig. 1c). The following evidence indicates strongly that this component is the α subunit of pyruvate dehydrogenase phosphate: (1) The *R_f* of the peak (0.45) was the same as the α subunit of purified pig heart ³²P-pyruvate dehydrogenase phosphate; little or no incorporation of ³²P was found corresponding to the other subunits of the complex (Fig. 1, a and b). Components of the pig heart complex and fat pad mitochondria have also been separated by SDS–polyacrylamide electrophoresis on adjacent tracks of a polyacrylamide slab. Incorporation of ³²P into protein components from fat pad mitochondria with *R_f* values corresponding to the β subunit (*R_f* 0.52), dihydrolipoate transacetylase (*R_f* 0.29) and dihydrolipoate dehydrogenase (*R_f* 0.36) was less than 3% of the incorporation into the peak which migrates as the α subunit (*R_f* 0.45) of pig heart pyruvate dehydrogenase. Similar results have been found using mitochondria from rat heart, liver and kidney incubated with succinate as substrate. (2) Maximum incorporation of ³²P into the peak was equivalent to 0.6–0.8 nmol phosphate per unit pyruvate dehydrogenase phosphate (Fig. 2). This level of incorporation is close to that observed with preparations of pyruvate dehydrogenase purified from pig heart³. (3) Treatment of phosphate buffer extracts of fat pad mitochondria with partially purified pig heart pyruvate dehydrogenase phosphatase¹¹ in the presence of Mg²⁺ and Ca²⁺ before precipitation of the proteins with perchloric acid resulted in complete disappearance of ³²P incorporation into the peak (*R_f* 0.45). (4) Incubation of fat pad mitochondria with pyruvate (2 mM) in the presence of oxoglutarate, malate and phosphate leads to a large decrease (by 70–80%) in the mitochondrial content of pyruvate dehydrogenase phosphate because pyruvate is a specific inhibitor of the kinase¹⁴. In these conditions, the

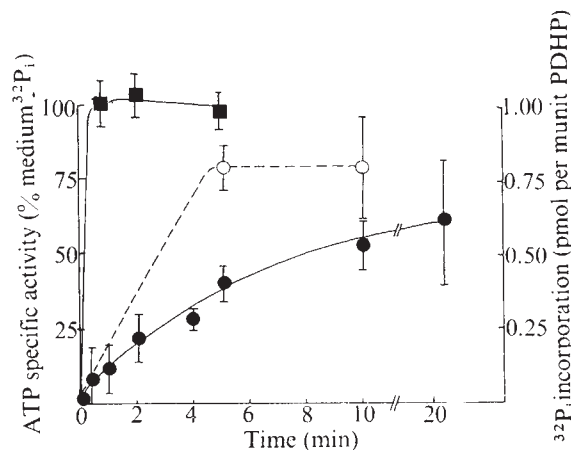


Fig. 2 Time courses of ³²P incorporation into the α subunit of pyruvate dehydrogenase phosphate (●, ○) and γ -³²P-ATP specific activity (■) in fat pad mitochondria incubated with ³²P_i. Mitochondria were prepared from fat pads incubated in the presence (---) or absence (—), of insulin, 10 mU ml⁻¹. After precipitation of the mitochondria in medium containing phosphate, oxoglutarate and malate for 3 min, ³²P_i was added to give 0.2 mM and a final specific activity of 1,000 to 5,000 c.p.m. pmol⁻¹ and incubation continued for various times (see text for details). After SDS–polyacrylamide electrophoresis as described in Fig. 1, the gels were cut into transverse slices (5 mm), dissolved in hydrogen peroxide (30% w/v) and then neutralised with 1 N HCl. Samples were taken up in a methoxy-ethanol-based scintillation fluid¹¹ for determination of radioactivity. In all cases a single peak of radioactivity with an *R_f* close to 0.45 was found (see Fig. 1c); incorporation into the α subunit was taken as the radioactivity in this peak with a correction (<5%) for background radioactivity taken as the mean of values in adjacent slices. Values are expressed as pmol of medium ³²P_i incorporated per unit of pyruvate dehydrogenase phosphate. Specific activity of intramitochondrial γ -³²P-ATP was measured by the method of England and Walsh¹³ modified for small concentrations of ATP (about 5 μ M). For these measurements, after incubation with ³²P_i for varying times, the mitochondria were centrifuged through an oil layer (silicone oil MSS60: dinonyl phthalate (3:2)) into perchloric acid (3%) containing glycerol (25% w/v). Each value is the mean $\pm$ s.e.m. of observations made on three to eight separate mitochondrial preparations.

maximum incorporation of ³²P_i into the peak (*R_f* 0.45) was decreased in three separate preparations of mitochondria by a very similar percentage. Closely parallel decreases in ³²P_i incorporation and pyruvate dehydrogenase phosphate content were also apparent on incubating mitochondria in the presence of uncoupler (FCCP, 1 μ M).

The incorporation of ³²P_i into the α subunit took 10–15 min to reach completion in mitochondria from control tissues. In mitochondria from insulin-treated tissue, incorporation seemed complete by 5 min (Fig. 2). The maximum incorporation (0.6–0.8 nmol phosphate per unit pyruvate dehydrogenase phosphate) was not significantly affected by insulin treatment. The specific activity of mitochondrial ATP was found to rise to that of the medium phosphate within 30 s (Fig. 2). Consequently the rate of incorporation of ³²P_i into the α subunit gives an estimate of the rate of the kinase reaction within the mitochondria, using the present conditions where no changes in the phosphorylation state of pyruvate dehydrogenase complex are occurring. The rate was found to be significantly greater in mitochondria from insulin-treated tissues. The increase in incorporation after 5 min was $44 \pm 11\%$ if results were expressed in terms of total pyruvate dehydrogenase activity and $36 \pm 9\%$ if expressed in terms of mitochondrial glutamate dehydrogenase (mean $\pm$ s.e.m. for seven separate paired preparations of mitochondria). In two experiments, measurements were also made after incubation for 2 min and again increases of incorporation (by 17 and 29%) were observed.

These results provide strong evidence against the hypothesis^{7,8} that the increase in the non-phosphorylated form of pyruvate dehydrogenase in adipose tissue exposed to insulin is brought

about by a decrease in kinase activity. Moreover, measurements of some of the known effectors of the kinase (ATP, ADP, NAD, NADH, acetyl CoA, CoA) have been made in mitochondria from control and insulin-treated tissue (refs 10, 15 and unpublished work with B. J. Bridges), but no clear evidence has been found for any changes which could result in a decrease in kinase activity. In particular, no reduction in the ATP:ADP concentration ratio has been observed. We conclude that it is more likely that the effect of insulin is mediated through an increase in the activity of the phosphatase. The subsequent increase in the active non-phosphorylated form of pyruvate dehydrogenase may then result in the enhanced rate of phosphorylation observed in the present studies. Since no differences in the activity of pyruvate dehydrogenase phosphatase persist in extracts of mitochondria from control and insulin-treated tissue¹⁶, insulin may act through changes in the mitochondrial concentration of one or more effectors of the phosphatase.

The only two effectors of the phosphatase that have been recognised to date are Mg^{2+} and Ca^{2+} which both activate the enzyme^{1,3,6} and so the strong possibility remains that the effects of insulin may be brought about by an increase in the free concentration within adipose tissue mitochondria of one or both of these divalent metal ions.

This work was supported by a grant from the MRC. We are grateful to Dr P. J. England and Professor P. J. Randle for advice and discussion.

W. A. HUGHES
R. M. DENTON

Department of Biochemistry,
University of Bristol Medical School,
Bristol BS8 1TD, UK

Received August 5; accepted October 13, 1976.

- ¹ Linn, T. C., Pettit, F. K., and Reed, L. J., *Proc. natn. Acad. Sci. U.S.A.*, **62**, 234-241 (1969).
- ² Barrera, C. R., et al., *Archs Biochem. Biophys.*, **148**, 343-358 (1972).
- ³ Denton, R. M., Randle, P. J., and Martin, B. R., *Biochem. J.*, **128**, 161-163 (1972).
- ⁴ Cooper, R. H., Randle, P. J., and Denton, R. M., *Nature*, **257**, 808-809 (1975).
- ⁵ Pettit, F. H., Pelley, J. W., and Reed, L. J., *Biochem. biophys. Res. Commun.*, **65**, 575-582 (1975).
- ⁶ Denton, R. M., et al., *Molec. cell. Biochem.*, **9**, 27-53 (1975).
- ⁷ Taylor, S. I., and Jungas, R. L., *Archs Biochem. Biophys.*, **164**, 12-19 (1974).
- ⁸ Weiss, L., Löffler, G., and Wieland, O., *Hoppe-Seyler's Z. physiol. Chem.*, **255**, 363-377 (1974).
- ⁹ Severson, D. L., Denton, R. M., Bridges, B. J., and Randle, P. J., *Biochem. J.*, **154**, 209-223 (1976).
- ¹⁰ Denton, R. M., *Proc. Nutr. Soc.*, **34**, 217-224 (1975).
- ¹¹ Severson, D. L., Denton, R. M., Pask, H. T., and Randle, P. J., *Biochem. J.*, **140**, 225-237 (1974).
- ¹² Weber, K., and Osborn, M., *J. biol. Chem.*, **244**, 4406-4412 (1969).
- ¹³ England, P. J., and Walsh, D. A., *Analyt. Biochem.*, (in the press).
- ¹⁴ Martin, B. R., Denton, R. M., Pask, H. T., and Randle, P. J., *Biochem. J.*, **129**, 763-773 (1973).
- ¹⁵ Denton, R. M., et al., *Proc. V International Congress of Endocrinology, Hamburg, 1976* (Excerpta Medica, in the press).
- ¹⁶ Stansbie, D., Denton, R. M., Bridges, B. J., Pask, H. T., and Randle, P. J., *Biochem. J.*, **154**, 225-236 (1976).

Circular and elongated linear forms of measles virus nucleocapsid

PARAMYXOVIRUSES are assuming increasing importance as possible causative agents of several human diseases^{1,2}. To date little is known about their mode of replication, although it has been suggested that the nucleocapsid replicates without extensive dissociation of the protein from the nucleic acid component³. Previous reports on the structure of nucleocapsids extracted from virions or infected cells, with or without further purification, have shown by electron microscopy that the molecules are linear, and although short pieces can be found, the majority are about 1.0 μ m long⁴. We report here the occurrence of rare circular forms of measles nucleocapsid as well as linear molecules of greater than normal length.

Three strains of measles virus have been examined; a non-haemagglutinating strain⁵ (P9), a haemagglutinating strain (TC243) and measles virus isolated during a transfusion experiment using DNA extracted from the brain of a multiple sclerosis patient (H.V.T. and Haire, unpublished). The origin of this virus (provisionally designated Miller virus), has not yet been established. The viruses were

grown in human embryonic lung (HeLu) and Vero cell monolayers which were infected with virus (about 1 plaque-forming unit per cell) by adsorption for 1 h at 37 °C and cultured in 199 medium + 10% calf serum at 37 °C for various times. Material for electron microscopy was prepared, with equivalent results, by lysing monolayers with 2% phosphotungstic acid or by rubbing off cells into the

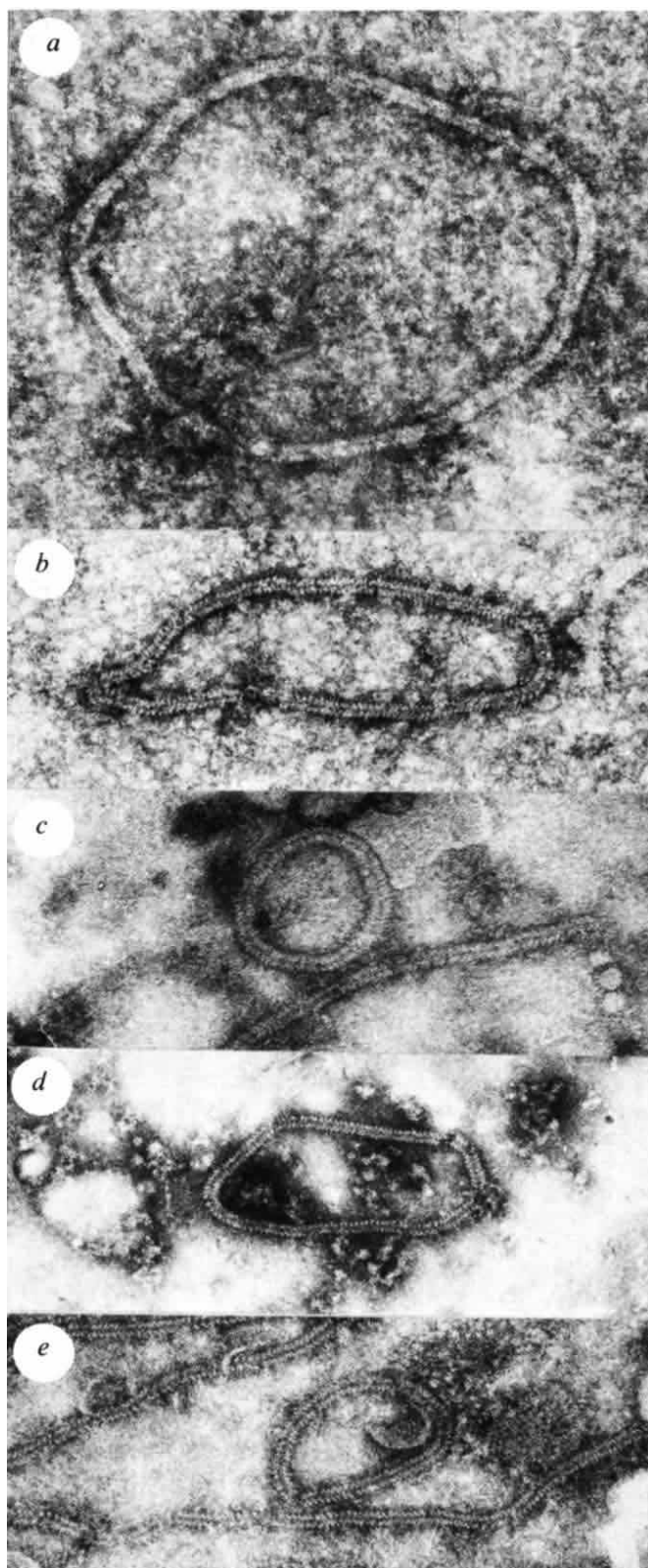


Fig. 1 Circular nucleocapsids of different sizes: a, b, and c, Miller virus grown in HeLu cells 1.5, 1.1 and 0.5 μ m respectively; d, TC243 virus grown in HeLu cells, 0.6 μ m; e, P9 virus grown in Vero cells, 0.5 μ m. $\times 125,000$.

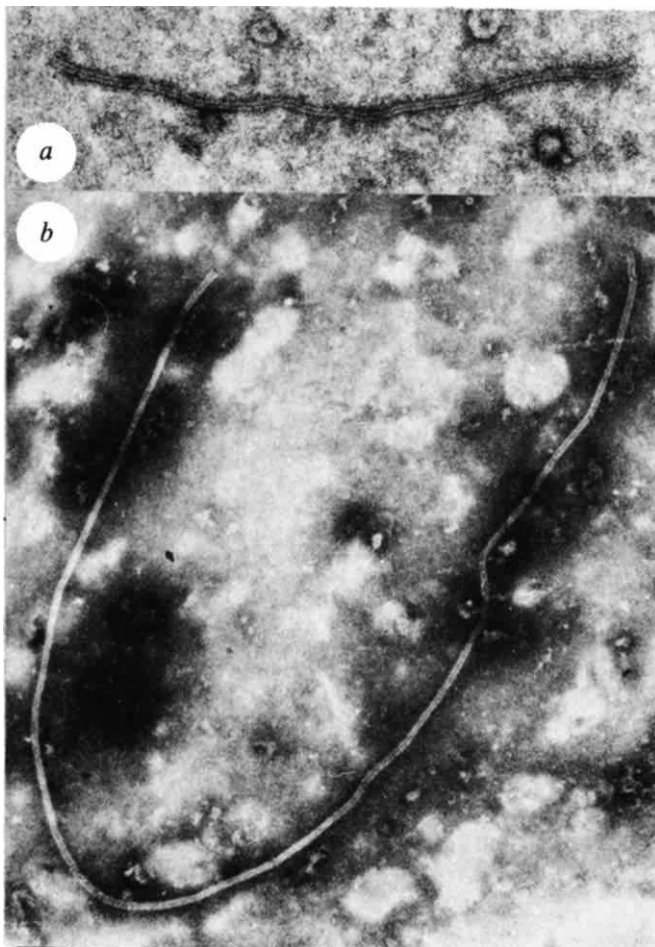


Fig. 2 Linear nucleocapsids of different lengths: *a*, Miller virus grown in HeLu cells, 1.2 μm ; *b*, P9 virus grown in HeLu cells, 3.6 μm . $\times 60,000$.

medium followed by centrifugation at 30,000 r.p.m. for 1 h with resuspension of the pellet in phosphate-buffered saline (PBS), or by washing monolayers with distilled water followed by Dounce homogenisation and low speed centrifugation to remove debris. The preparations, without further purification, were put on to carbon-Formvar coated grids, excess solution was removed with filter paper and, in the latter two methods, grids were then stained with 2% phosphotungstic acid. Grids were examined immediately in an AEI Corinth 500 electron microscope.

Unbroken circular nucleocapsids were found in all three virus strains and both cell cultures (Fig. 1), but were rare compared to the linear forms. The size of the circles varied between 0.2 and 1.5 μm and could be found in preparations from cultures from 18 h post-infection onwards, that is, at the normal time of nucleocapsid accumulation⁶. Present techniques do not indicate whether the number of circles varies during the growth cycle of the virus. In addition to unbroken molecules a small proportion of molecules showing one or more breaks or discontinuities were observed. It is not possible to ascertain whether these breaks are real or artefacts of preparation. No rings or linear molecules were found in uninfected cultures.

The most frequently found length of linear nucleocapsid was about 1.1 μm (Fig. 2*a*), but we also observed abnormally long nucleocapsids (Fig. 2*b*) in all three virus strains with one of nine times this length in Miller virus grown in HeLu cells. Long molecules seem to increase in number and length with time after infection, particularly in HeLu cells in which the cytopathic effect is less destructive

permitting observation for times up to 3 weeks post-infection. Although no circular molecules have been described previously for any paramyxovirus, rare nucleocapsid molecules of 2 and 4 μm lengths which were considered to be end-to-end aggregates of normal length nucleocapsids have been reported for the parainfluenza virus SV5 (ref. 2).

Our results do not allow any conclusions as to the origin and function of either the circular or the long molecules, but they may indicate, assuming that the molecules contain RNA, that there are circularisation processes for RNA; evidence for this has recently been presented in an oncornavirus system⁷. Since no ligase for RNA is known, we speculate that circles arise from the presence of complementary ends on some or all of the RNA molecules. The long molecules could result from end-to-end linkage of similar RNA molecules.

Although it cannot be ruled out that the circles are accidental forms of nucleocapsid with no role in the normal replication process, nevertheless, we suggest two possible functions for these molecules. First, that the circles represent the templates from a replicative intermediate arising during a "rolling circle" type of replication. Such a model could also provide an alternative explanation for the presence of long molecules. The varying size of the circles could be accounted for by the presence of replicating defective genomes. Second, the circles are encapsidated RNA molecules which function as templates for DNA replication (presumably by a reverse transcriptase) ultimately producing circular double-stranded DNA molecules which could be in suitable form for integration into the cell genome. This would provide a mechanism to explain the existence of infectious DNA molecules which have been obtained from cultures persistently infected with measles⁸. There may be a relationship between such a mechanism and the integration of virus-specific sequences which has been suggested to occur in diseases such as systemic lupus erythematosus and multiple sclerosis⁸.

It will be interesting to see whether similar forms of nucleocapsid can be identified in other RNA virus systems.

This work was supported by an MRC Programme grant. We thank Mr G. Clarke and Mr T. McLaughlin for assistance.

H. V. THORNE
EVELYN DERMOTT

*Department of Microbiology and Immunobiology,
The Queen's University of Belfast,
Grosvenor Road,
Belfast BT12 6BN, UK*

Received September 20; accepted October 5, 1976.

- ¹ Connolly, J. H., Allen, I. V., Hurwitz, L. S., and Millar, J. H., *Lancet*, i, 542-544 (1967).
- ² *Multiple Sclerosis Research* (HMSO, London, 1975).
- ³ Kingsbury, D. W., *Med. Microbiol. Immun.*, **160**, 73-83 (1974).
- ⁴ Compans, R. W., and Choppin, P. W., *Proc. natn. Acad. Sci. U.S.A.*, **57**, 949-956 (1967).
- ⁵ Shirodaria, P. V., Dermott, E., and Gould, E. A., *J. gen. Virol.*, **33**, 107-115 (1976).
- ⁶ Nakai, M., and Imagawa, D. T., *J. Virol.*, **3**, 187-197 (1969).
- ⁷ Collett, M. S., and Faras, A. J., *Proc. natn. Acad. Sci. U.S.A.*, **73**, 1329-1332 (1976).
- ⁸ Zhdanov, V. M., *Nature*, **256**, 471-473 (1975).

RNA homology between subacute sclerosing panencephalitis and measles viruses

VIROLOGICAL and immunological studies in subacute sclerosing panencephalitis (SSPE) have associated this disease with measles virus^{1,2}. Measles-like virus (referred to as SSPE virus) has been isolated from brain material of patients with SSPE and all SSPE patients reveal an hyperimmune reaction to measles virus antigen. In spite of these major findings the aetiology and pathogenicity of SSPE is still not understood, and the rarity and rural prevalence of this disease

remain unexplained³. If measles virus is involved then additional factors either host or virus dependent, must determine the disease since the occurrence of measles virus and its acute infection cannot be correlated with SSPE. So far, no major host factor, either immunological or genetic, has been discovered which would answer these questions⁴.

Biological and ultrastructural comparisons of SSPE and measles viruses have indicated some differences⁵⁻⁷. The present communication compares several SSPE isolates with measles virus in terms of genome homology. The genome of measles and SSPE viruses consists of a single-stranded piece of RNA which has a sedimentation coefficient of 50S (refs 8 and 9). After infection this RNA is transcribed into complementary messenger (m)RNA molecules which sediment at approximately 18S in sucrose gradients. The genome homology of different viruses can be compared simply by hybridising either the mRNAs of different viruses to one radioactive labelled genome or vice versa. One problem with hybridisation experiments involving SSPE viruses is that in the early passage levels after isolation, large amounts of defective genomes are produced. These RNAs contain some of the sequences of the infectious genome and have the same sedimentation coefficients as the virus messengers^{10,11}. Thus, if "18S" RNAs from infected cells are used in hybridisation experiments they will "self-hybridise". Such a background of self-hybridisation would give erroneous results in any hybridisation experiments. The mRNAs were therefore purified on the basis of their poly(A) content by chromatography on oligo d(T)-cellulose. A second problem is the difficulty in producing a complete set of highly labelled mRNAs or genome RNAs because of the long and variable growth rates of these viruses. Therefore we have used a competition-hybridisation method where only one or two RNAs need to be labelled and the different growth rates are unimportant.

The viruses compared in the present study were two measles viruses (the attenuated Edmonston strain and Woodfolk, a wild measles strain), three strains of SSPE (Mantooth, Lec and Jac), the immunologically related Onderstepoort strain of canine distemper virus (CDV) and a bovine-meningo encephalitis virus (107 virus). The latter virus has been isolated from the brain of a calf which had died from a meningo-encephalomyelitis, and on the basis of its morphological and biological characteristics belongs to the measles-CDV-Rinderpest virus group¹². The RNA of parainfluenza 1 6/94 virus and Vero cell ribosomal RNA were used as controls.

All viruses were grown in Vero cells except 107 virus which was cell associated with BHK cells, and mRNAs were isolated between 18 and 60 h after infection. In the competition hybridisation experiments we selected two viruses: the Edmonston and Mantooth strains as prototypes of measles and SSPE respectively. Unlabelled genome RNA was prepared from purified nucleocapsids as previously described³. The corresponding mRNAs of these two viruses were labelled with ³H-uridine by adding large quantities (100 μ Ci ml⁻¹) of isotope to actinomycin D-treated infected cells. The mRNAs were extracted and purified by sucrose-gradient sedimentation and oligo d(T)-cellulose chromatography (Fig. 1). Unlabelled mRNAs of all the viruses were then purified from infected cells using the same method. The genetic relatedness or comparative homology of the viruses could be measured by the ability of the unlabelled or "cold" mRNAs to compete with the hybridisation of the labelled mRNA to its corresponding genome.

A typical set of results obtained is shown in Figs 2 and 3. Figure 2 shows the competition profiles of the mRNAs against the hybridisation of ³H-labelled Edmonston mRNA to its genome. It can be seen that the Woodfolk strain of measles virus shares 100% homology with the Edmonston. Similarly, the three SSPE viruses tested shared 100% homology with Edmonston measles. CDV and 107 virus shared

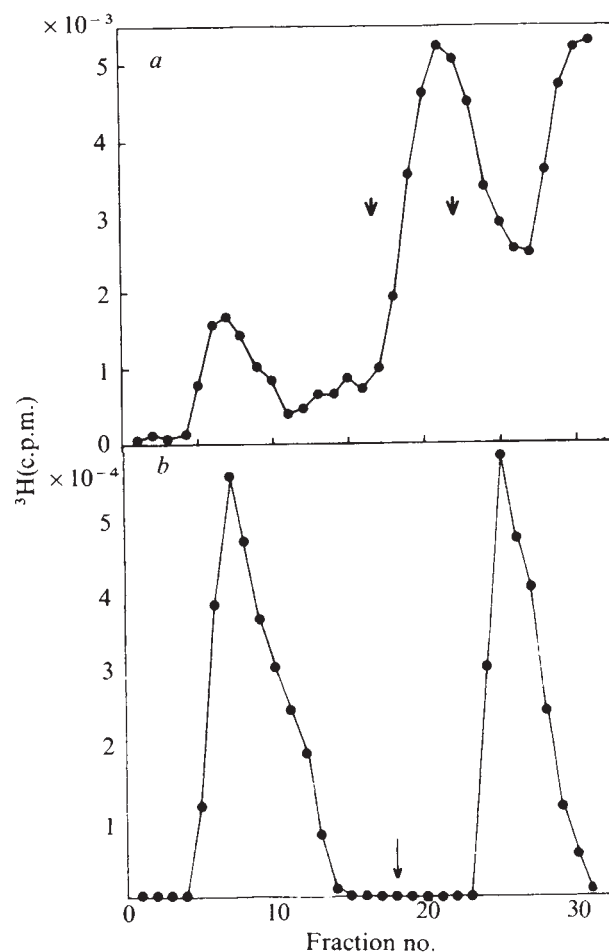


Fig. 1 Purification of virus-specific mRNAs. RNA was extracted from infected cells by the following procedure. Cells were washed twice with ice-cold NTE buffer (0.01 M Tris-HCl (pH 7.5), 0.1 M NaCl, 0.001 M EDTA), scraped into fresh NTE buffer and collected by centrifugation at 2,000g for 10 min at 4 °C. Pellets were resuspended in sterilised distilled water (0.1 ml) and allowed to swell on ice for 15 min. Cells were disrupted by douncing 10 strokes in a tight-fitting glass homogeniser, and nuclei were removed by centrifugation at 800g for 8 min. Supernatants were removed, made 2% in sodium dodecyl sulphate (SDS) and heated at 56 °C for 2 min. Released RNA was directly sedimented on 35 ml linear 15 to 30% (w/w) sucrose gradients in NTE buffer containing 0.5% (w/v) SDS. Gradients were centrifuged for 16 h at 20,000g at 20 °C. After centrifugation, fractions were collected from the bottom of the tube using an LKB pump and an ISCO fractionator which measured absorbance continuously at 254 nm. Aliquots (25 μ l) of each fraction were precipitated with 3 volumes of 10% (w/v) trichloroacetic acid (TCA). Precipitates were collected using a Millipore Manifold 3025 filtration apparatus, and radioactivity was counted in Bray's solution. A typical radioactivity profile for Edmonston-measles virus is shown in *a*. RNA which sedimented at 12 to 36S (fractions 25-14) was pooled and precipitated overnight in 2 volumes of ethanol at -20 °C. Precipitates were collected by centrifugation at 8,000g for 30 min and allowed to dry at room temperature for 20 min. Samples were dissolved in TE buffer (0.01 M Tris-HCl (pH 7.4), 1.5 M LiCl, 0.001 M EDTA), allowed to stand at 4 °C for 24 h and then centrifuged at 12,000g for 25 min. The pellets which contained double-stranded RNA intermediates were discarded and the supernatants (predominantly single-stranded RNA) were precipitated in 2 volumes of ethanol as described above. Precipitates were collected, dissolved in 0.01 M Tris-HCl (pH 7.5), 0.5 M NaCl, 0.001 M EDTA containing 0.1% (w/v) SDS (binding buffer) and applied to 0.4 g of oligo d(T)-cellulose in a disposable pipette column. The column was washed with 8 ml of binding buffer and bound RNA was eluted with 0.005 M Tris-HCl (pH 7.4), 0.001 M EDTA containing 0.1% (w/v) SDS. Radioactivity levels were determined (Fig. 1*b*) and the purified mRNA precipitated with ethanol. The arrows in *a* represent the sedimentation position of Vero cell 28S and 18S ribosomal RNA; in *b* the arrow indicates the change of buffer from binding to elution.

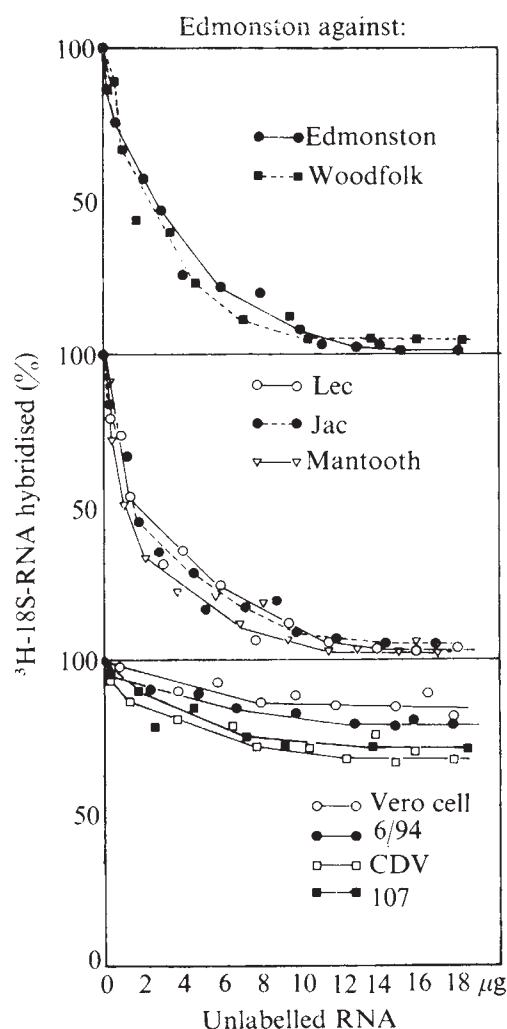


Fig. 2 Competition of unlabelled mRNAs on the hybridisation of Edmonston virus genome to its mRNA. Samples of Edmonston 50S RNA, ^3H -uridine labelled (12,000–14,000 c.p.m.) Edmonston mRNA and various amounts of unlabelled mRNAs were mixed in $2\times\text{SSC}$ ($\text{SSC} = 0.15\text{ M}$ sodium citrate, 0.15 M NaCl) containing 0.05% (w/v) SDS. Mixtures (final vol. $150\text{ }\mu\text{l}$) were added to glass tubes ($0.2\times 6.0\text{ cm}$). The tubes were briefly centrifuged to allow complete mixing of the RNAs and then sealed. After denaturation by boiling for 4 min hybridisation was allowed to occur by incubation at 60°C for 12–14 h. Tubes were cooled on ice, opened and the contents treated with ribonucleases (pancreatic $50\text{ }\mu\text{g ml}^{-1}$, T_1 $8\text{ }\mu\text{g ml}^{-1}$) at 37°C for 30 min. The reaction was stopped by the addition of 3 volumes of 10% (w/v) TCA and acid-insoluble radioactivity determined.

30% homology with measles virus, whereas parainfluenza 1 6/94 virus mRNA and Vero cell ribosomal RNA provided only a low and inconsistent level of competition. Figure 3 shows an identical experiment where the viruses were compared with the Mantooth strain of SSPE virus. It can be seen that all three strains of SSPE virus share 100% homology. However, in sharp contrast to the previous data the two measles virus strains were able only to compete out approximately 90% of the labelled SSPE mRNAs. It would therefore appear that, whereas all the genetic information contained in the measles genome is present in SSPE virus, the latter contains an additional piece of genetic information. CDV and 107 virus shared 38% homology with Mantooth SSPE, an increase in genetic relatedness to that observed with Edmonston measles virus. The parainfluenza 1 6/94 and Vero cell RNAs showed again only a low and inconsistent degree of relatedness. A summary of the percentage homologies of the different viruses to the measles and SSPE prototypes is shown in Table 1.

Table 1 Summary of competition-hybridisation results*

To:	% Similarity of	
	Edmonston	Mantooth
Edmonston	100	91
Woodfolk	97	88
Mantooth	99	100
Lec	98	102
Jac	99	99
CDV	29	43
107 Virus	28	41
6/94	19	18
Vero cell RNA	16	12

*The values represent the means of three individual experiments.

These results show that, whereas all the genetic information of measles virus is contained in SSPE viruses, the latter apparently contain an additional 10% information.

These results are in disagreement with the direct hybridisation experiments of Yeh⁸ who reported that the Woodfolk strain of measles virus contained only 60% of the genetic information of the SSPE-Lec virus genome. But in this work purified mRNAs were not used, saturation levels of annealing were not obtained, and the genetic relationship of the SSPE to the measles genome was not described.

An intriguing explanation for the results obtained would be that the viruses of SSPE may arise as a recombination

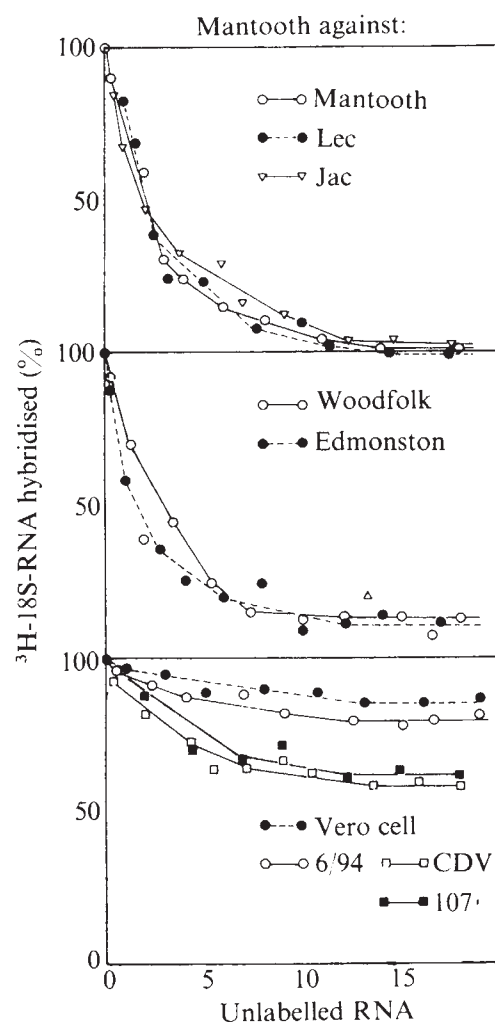


Fig. 3 Competition of unlabelled mRNAs to the hybridisation of Mantooth virus genome to its mRNA. Details were as described in Fig. 2. Each sample contained 16,000 c.p.m. of ^3H -uridine-labelled Mantooth mRNA.

of measles virus with another as yet unknown second virus. The additional information represents a molecular weight in the order of $4\text{--}5 \times 10^5$ which would have a sedimentation of about 12S. Defective RNAs of this size have been reported for paramyxoviruses including measles^{11,14}. Therefore it would seem likely that if a recombination occurs then it is with the intact genome of measles virus and a defective genome of the second virus.

The data exclude the possibility that the genomes of the SSPE viruses tested in the present study have arisen from a recombination between a complete and defective measles RNA genome, since the mRNA corresponding to the additional genomic information of SSPE is not present in measles-infected cells. The formation of covalently linked recombinant genomes is neither genetically nor biologically impossible. Recent work with vesicular stomatitis virus (VSV) has shown that mixed infections with defective and infectious viruses can result in the production of genetically stable recombinant genomes¹⁴ and a state of virus persistence *in vitro*¹⁵. A similar mechanism of mixed infection would account for the slow process of infection and the accompanying persistence observed in SSPE.

If such a double infection/recombination does occur in SSPE, it would account for two important features of the disease. Firstly, the rarity of the disease would be consistent with the low possibility of a mixed infection resulting in genetic recombination. Secondly, if the second virus is an animal RNA virus then it would account for the predominant rural distribution of the disease.

This work was supported by the Deutsche Forschungsgemeinschaft. We thank Dorothea Müntjes, Ingeborg Euler and Heidi Zehnter for technical assistance. The discussions with Dr H. Schulte-Holthausen, Institut für klinische Virologie, Universität Erlangen, are gratefully acknowledged.

WILLIAM W. HALL
VOLKER TER MEULEN

Institute of Virology and Immunobiology,
University of Würzburg,
Versbacher Landstrasse 7,
8700 Würzburg, FRG

Received August 2; accepted September 29, 1976.

- ¹ Connolly, J. H., Allen, I. V., Hurwitz, L. J., and Millar, J. H. D., *Lancet*, i, 542–544 (1967).
- ² Meulen, V. ter, Katz, M., and Muller, D., *Curr. Top. Microbiol. Immun.*, 57, 1–38 (1972).
- ³ Brody, J. A., and Detels, R., *Lancet*, ii, 500–501 (1970).
- ⁴ Kreth, H. W., Kaeckell, Y. M., and Meulen, V. ter, *Med. Microbiol. Immun.*, 160, 191–199 (1974).
- ⁵ Payne, F. E., and Baublis, J. V., in *Perspectives in Virology*, 7, 179–185 (Academic, New York, 1971).
- ⁶ Meulen, V. ter, Katz, M., Oyanagi, S., Barbanti-Brodano, G., and Koprowski, H., *J. infect. Dis.*, 126, 11–17 (1972).
- ⁷ Katz, M., *Med. Microbiol. Immun.*, 160, 247–250 (1974).
- ⁸ Yeh, J., *J. Virol.*, 12, 962–967 (1973).
- ⁹ Hall, W. W., and Martin, S. J., *J. gen. Virol.*, 19, 175–188 (1973).
- ¹⁰ Kiley, M. P., Gray, R. H., and Payne, F. E., *J. Virol.*, 13, 721–728 (1974).
- ¹¹ Hall, W. W., Martin, S. J., and Gould, E. A., *Med. Microbiol. Immunol.*, 160, 155–164 (1974).
- ¹² Bachmann, P. A., et al., *Archs Virol.*, 48, 107–120 (1975).
- ¹³ East, J. L., and Kingsbury, D. W., *J. Virol.*, 8, 161–173 (1971).
- ¹⁴ Lazzarini, R. A., Weber, G. H., Johnson, L. D., and Stammering, G. J., *J. molec. Biol.*, 97, 289–307 (1975).
- ¹⁵ Holland, J. J., *Scient. Am.*, 230, 33–40 (1974).

Interferon-mediated protein kinase and low-molecular-weight inhibitor of protein synthesis

PROTEIN synthesis in cell-free systems from mouse L-cells pretreated with the antiviral agent interferon¹ shows an enhanced sensitivity to inhibition by double-stranded RNA (dsRNA)^{2,3}. The possible significance of this with respect to events in intact interferon-treated virus-infected cells has already been discussed^{2,4}. We have already shown that

the addition of small amounts of a post-ribosomal supernatant fraction from interferon-treated cells (interferon cell sap) renders protein synthesis in control cell-free systems sensitive to inhibition by dsRNA⁴. Our results are in accord with a two-step model involving an initial 100-fold activation of an inhibitor on incubation of the interferon cell sap with ATP and dsRNA (activation step), the activated inhibitor then interacting with the protein-synthetic system to inhibit translation (inhibitory step)⁵. In view of the dependence of the inhibition of protein synthesis upon incubation with ATP as well as dsRNA⁵ it was of interest to determine if a protein kinase(s) is involved, as seems to be the case in reticulocyte lysates in which phosphorylation of the initiator Met-tRNA binding factor (IF-E2) has been implicated following incubation of the lysates under a variety of inhibitory conditions including the presence of dsRNA (P. Farrell,

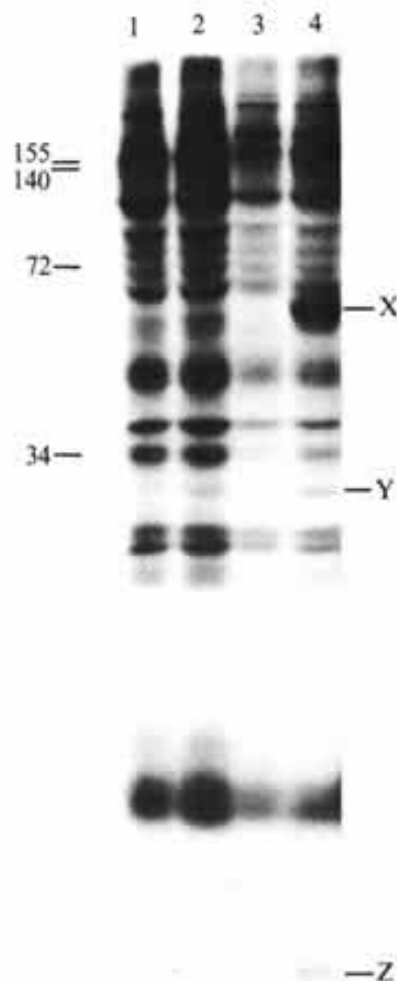


Fig. 1 A dsRNA-dependent protein kinase activity in cell sap from interferon-treated cells: electrophoretic analysis of phosphorylated polypeptides. Control cell sap minus (1) and plus (2) dsRNA. Interferon cell sap minus (3) and plus (4) dsRNA. Each cell sap (10 μ l) was incubated with 0.1 mM γ -³²P-ATP (8 Ci mmol⁻¹, Radiochemical Centre) plus or minus *Penicillium chrysogenum* phage dsRNA² (400 ng ml⁻¹) in a final volume of 13 μ l. Incubation was for 40 min at 30 °C. Material equivalent to 5 μ l of each incubation was analysed by electrophoresis on a 10% polyacrylamide slab gel containing 0.1% sodium dodecyl sulphate (SDS)^{8,9}. An autoradiograph of the stained, dried gel is shown. The numbers to the left give the molecular weights in thousands of the major reovirus polypeptides λ 1, λ 2, μ 2 and σ 3¹⁰, included as markers. Interferon treatment of mouse L-cells with low doses (5 to 20 effective units ml⁻¹) of highly purified mouse interferon ($\geq 5 \times 10^7$ reference units mg⁻¹ protein) and the preparation of cell extracts and of the dialysed interferon and control cell saps were as described previously^{2,4}.

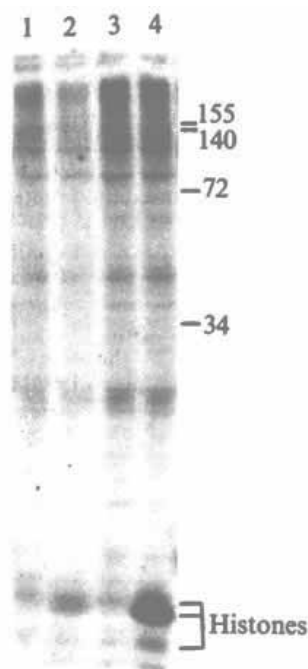


Fig. 2 Phosphorylation of histones by a dsRNA-dependent protein kinase in cell sap from interferon-treated cells: electrophoretic analysis of the phosphorylated histones. Control and interferon cell saps were first activated as in Fig. 1 but with unlabelled ATP (1 mM). Histone was then labelled by subsequent incubation of either the control cell sap (2 μ l) activated minus (1) or plus (2) dsRNA, or the interferon cell sap (2 μ l) activated minus (3) or plus (4) dsRNA, with 0.12 mM γ - 32 P-ATP (8 Ci mmol $^{-1}$) and 1 μ l of 2 mg ml $^{-1}$ calf thymus histone (type IIA, Sigma). Incubation was for 30 min at 30 °C in a total volume of 5 μ l. Material equivalent to one fourth of each incubation was analysed by electrophoresis as in Fig. 1. An autoradiograph of the stained, dried gel is shown. The molecular weight markers were as in Fig. 1.

and dsRNA, however, is heat stable and of relatively low molecular weight. It does not correspond either to the major polypeptide product of the interferon-dsRNA specific phosphorylation, or, presumably, to phosphorylated IF-E2.

To determine whether a protein kinase might be involved in the activation of the inhibitor formed in response to incubation of the interferon cell sap with dsRNA and ATP 5 , the incubation was carried out in the presence of γ - 32 P-ATP and the phosphorylated polypeptides were analysed by electrophoresis on polyacrylamide gels (Fig. 1). With interferon cell sap plus dsRNA (Fig. 1, channel 4) a major phosphorylated band (X) with an apparent molecular weight of about 60,000 (60K) and an additional band (Z), which migrated near the ion front, were observed which were not detected in corresponding controls. The phosphorylated polypeptide Y of approximately 30,000 (30K) in molecular weight was enhanced in the presence of dsRNA in both interferon and control preparations, but the increase was routinely greater in the interferon than the control (Fig. 1, channels 2 and 4).

K. Balkow, T. Hunt and R. J. Jackson, personal communication 6 . In accord with this, we report here the presence of a dsRNA-dependent protein kinase activity in interferon cell sap. The inhibitor of protein synthesis which is activated on incubation of interferon cell sap with ATP

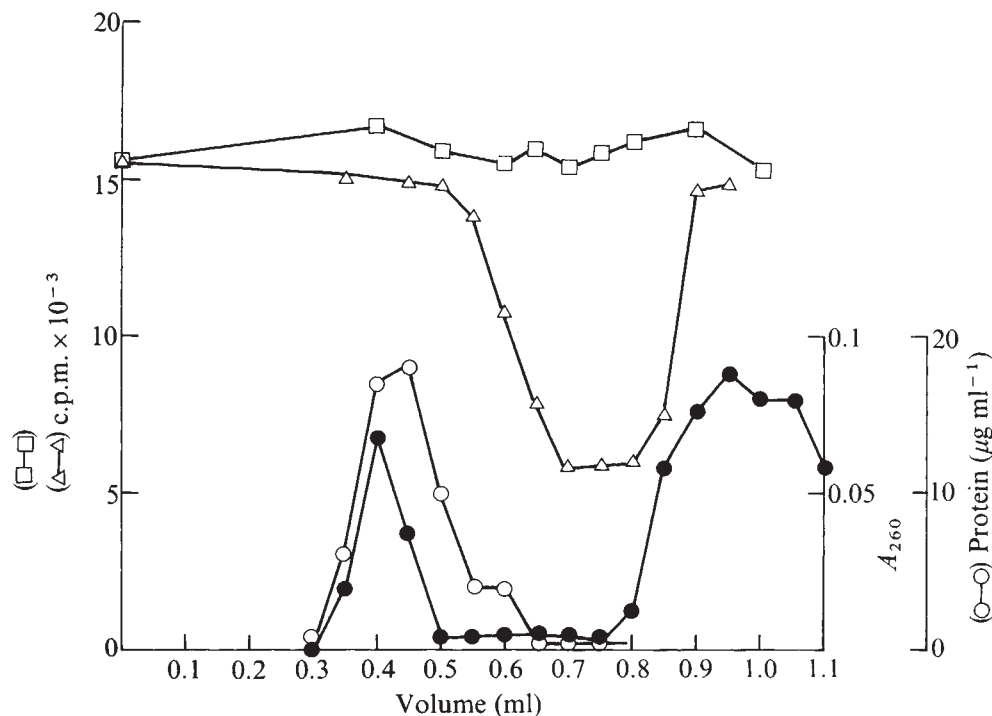


Fig. 3 Gel filtration chromatography of the activated inhibitor. Control and interferon cell saps were activated as in Fig. 1 but in the presence of unlabelled ATP (1 mM), heated to 90 °C for 5 min and centrifuged at 10,000 g for 10 min to remove denatured protein. Each supernatant (25 μ l) was fractionated by passage through a column (3 mm in diameter and 130 mm long) of Sephadex G-25 (Fine) equilibrated and eluted with 50 mM KCl and 20 mM Tris-hydrochloride (pH 7.6). Fractions (50 μ l) were collected and 2 μ l samples assayed for inhibitor activity on the translation of encephalomyocarditis virus (EMC) RNA in cell-free systems (25 μ l final volume) from control mouse L-cells 1,5,9 . The left hand ordinate gives the incorporation (c.p.m. per 10 μ l sample) of a 14 C-amino acid mixture into hot trichloroacetic acid-insoluble polypeptide in response to EMC RNA in the cell-free system 1,5,9 . Interferon (Δ) and control ($\square$) cell saps each activated in the presence of dsRNA. Interferon and control cell saps activated in the absence of dsRNA gave results essentially identical to the control plus dsRNA ($\square$). The right hand ordinates give the protein concentration 11 ($\circ$) and absorbance at 260 nm ($\bullet$) of the fractions obtained with the interferon cell sap which had been activated in the presence of dsRNA. The peak of optical density at 260 nm eluting between 0.8 and 1.1 ml represents primarily the ATP added for activation.

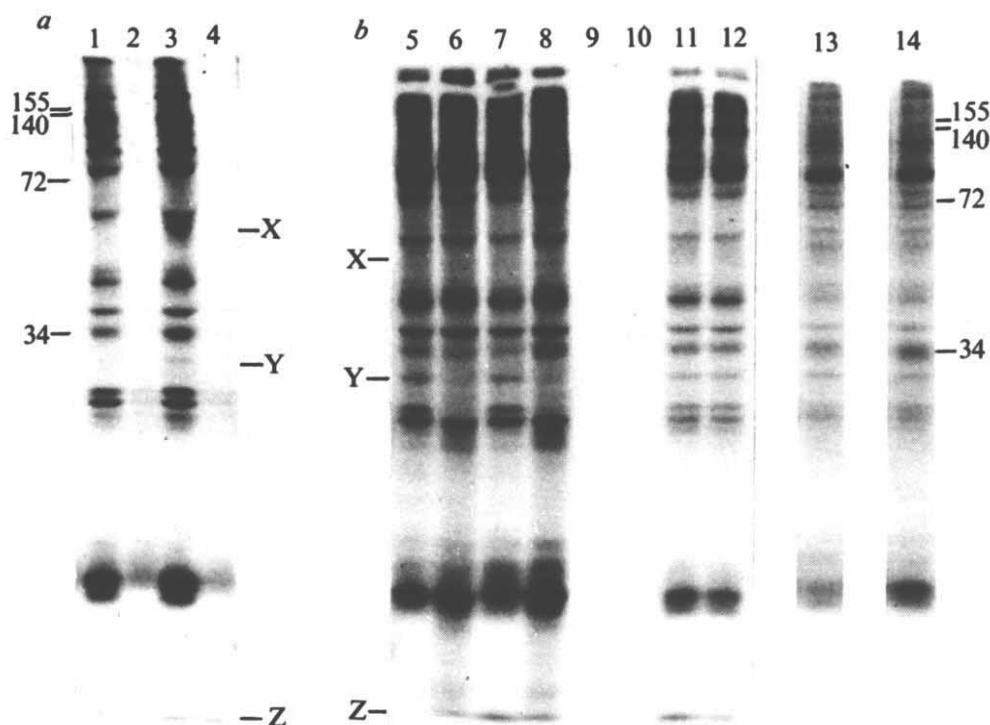


Fig. 4 *a*, Absence of phosphorylated polypeptides X and Y (Fig. 1) in the heat-stable inhibitor fraction. Interferon and control cell saps were incubated with γ - 32 P-ATP and dsRNA as in Fig. 1. A portion of each was heated to 90 °C for 5 min and centrifuged at 10,000*g* for 10 min. Samples (2 μ l) of the supernatant and of the original (unheated) activated material were analysed by electrophoresis on a polyacrylamide slab gel as in Fig. 1. Activated control cell sap (1) and supernatant after heating (2). Activated interferon cell sap (3) and supernatant after heating (4). *b*, Assay for protein kinase activity with activated inhibitor in the presence of histone, cell sap (S100), or post-mitochondrial supernatant (S10) fractions. Control and interferon cell saps were activated in the presence of dsRNA as in Fig. 1 but with unlabelled ATP (1 mM), heated to 90 °C for 5 min and centrifuged at 10,000*g* for 10 min. Samples (2 μ l) of each supernatant (interferon and control) were then incubated with γ - 32 P-ATP (2 μ l, 5 μ Ci μ l $^{-1}$, 8 Ci mmol $^{-1}$) in the presence of: (9 and 10) histone alone (1 μ l, 2 mg ml $^{-1}$ as in Fig. 2); (5 and 7) control cell sap alone (3 μ l); (6 and 8) control cell sap (3 μ l) plus histone (1 μ l); (11 and 12) interferon cell sap alone (3 μ l); (13 and 14) a post-mitochondrial supernatant (S10) fraction (3 μ l) from mouse L-cells which had been preincubated and dialysed in preparation for its use in the cell-free system 1,9 . Incubations for channels 7, 8, 10, 12 and 14 were with the supernatants from heated activated interferon cell sap, those for channels 5, 6, 9, 11 and 13 were with corresponding control material. Incubations were for 30 min at 30 °C. Samples representing one fourth of each incubation were analysed by electrophoresis on polyacrylamide slab gels 8,9 . Autoradiographs of the stained, dried gels are presented. The molecular weight markers were as in Fig. 1.

The appearance of these phosphorylated bands (Fig. 1, X, Y and Z) could reflect the presence of a new or increased kinase(s) or of new substrates (or both). As a test for an enhanced protein kinase activity, a potential substrate (calf thymus histone) was added to the interferon and control cell saps after activation and its phosphorylation examined by gel electrophoresis (Fig. 2). The marked increase, in the presence of interferon cell sap and dsRNA, in the phosphorylation of one of the major histone bands in particular (Fig. 2, channel 4) strongly suggests the activation of a specific kinase, although by no means excluding an additional or altered substrate as part of the explanation.

The major product of the interferon-dsRNA mediated kinase activity (X, Fig. 1) does not, however, appear to correspond to the inhibitor of protein synthesis activated on incubation of the interferon cell sap with ATP and dsRNA. This activated inhibitor is heat stable and seems to be of relatively low molecular weight. Thus after activation of the inhibitor by incubation of the interferon cell sap with dsRNA and ATP, heating to 90 °C for 5 to 15 min and centrifugation to remove the heat-denatured protein, the activated inhibitor is recovered *in toto* in the supernatant fraction. On passage of this supernatant through a column of Sephadex G-25 the inhibitor is retained separate from the peak of residual protein excluded from the column (Fig. 3). It seems unlikely,

therefore, that the activated inhibitor corresponds to either X or Y (Fig. 1). In fact, neither of these remain in the active supernatant on centrifugation after heating to 90 °C as above (Fig. 4, channels 1–4). (They can be recovered from the pellet, data not shown.)

Accepting that the activated inhibitor is neither X nor Y (Fig. 1), it was of interest to ask if a protein kinase is involved in the action of the activated inhibitor (inhibitory step). The heated activated inhibitor has no detectable kinase activity when assayed with histone as a substrate in the absence of added cell sap (Fig. 4, channels 9 and 10). Nor does it seem to function as an activator of protein kinase activity in cell sap or in the complete protein-synthetic system (Fig. 4, channels 5–8 and 11–14), although in the latter case a small quantitative difference in the 60K and 30K regions of the gel cannot be excluded (Fig. 4, channels 13 and 14). The differences in intensity of band Z here and in Fig. 1 are intriguing but variable and their significance is not yet clear.

There is, therefore, in cell sap from interferon-treated cells a dsRNA-dependent protein kinase(s) absent (or very much reduced) in corresponding controls. The main high-molecular-weight phosphorylated polypeptide products of this kinase(s) (X and Y, Fig. 1) do not, however, correspond to the heat-stable, low-molecular-weight inhibitor formed in response to incubation of interferon cell sap with ATP and dsRNA. Nor does the inhibition mediated by this

inhibitor seem to involve phosphorylation of these polypeptides. On the other hand, on a molecular weight basis alone, it is possible that a component of the band Z (Fig. 1) might be the active inhibitor, although our initial attempts to establish this have proved inconclusive. Certainly, until the biochemical nature of Z and of the activated inhibitor is known, the details of the activation and inhibitory steps worked out and the basis of the requirement for ATP understood, it would be premature to exclude a possible role for a protein kinase(s) in the inhibition of protein synthesis in these systems.

In more general terms, modulation of protein kinase activity by interferon may be of wider significance and the phosphorylation of histone (Fig. 2) could be of interest in this respect. It is conceivable that such kinases could be involved in the control of transcription or of other viral or cellular functions¹³ and thus account, in part at least, for the diversity of effects attributed to interferon.

This investigation was aided by a grant from the American Cancer Society to W.K.R. M.J.C. was the recipient of a Mr and Mrs John Jaffe (Royal Society) Fellowship. An interferon-dsRNA specific protein kinase activity has also recently been observed by P. Lengyel and M. Revel and their colleagues^{14,15}.

WALDEN K. ROBERTS*
ARA HOVANESSIAN
RONALD E. BROWN
MICHAEL J. CLEMENS†
IAN M. KERR

The National Institute for Medical Research,
London NW7 1AA, UK

Received August 31; accepted October 14, 1976.

*Present address: Department of Microbiology, University of Colorado Medical Center, Denver, Colorado, 80220, U.S.A.

†Present address: Department of Biochemistry, St. George's Hospital Medical School, Tooting, London SW17 0QT.

- 1 Finter, N. B. (ed.), *Interferon and Interferon Inducers*, 2nd ed. (North-Holland, Amsterdam, 1973).
- 2 Kerr, I. M., Brown, R. E., and Ball, L. A., *Nature*, **250**, 57-59 (1974).
- 3 Content, J., Lebleu, B., Nudel, U., Zilberstein, A., Berissi, H., and Revel, M., *Eur. J. Biochem.*, **54**, 1-10 (1975).
- 4 Kerr, I. M., Brown, R. E., Clemens, M. J., and Gilbert, C. S., *Eur. J. Biochem.*, **69**, 551-559 (1976).
- 5 Roberts, W. K., Clemens, M. J., and Kerr, I. M., *Proc. natn. Acad. Sci. U.S.A.*, **73**, 3136-3140 (1976).
- 6 Levin, D. H., Ranu, R. S., Ernst, V., Fifer, M. A., and London, I. M., *Proc. natn. Acad. Sci. U.S.A.*, **72**, 4849-4853 (1976).
- 7 Cox, R. A., Kanagalingam, K., and Sutherland, E. S., *Biochem. J.*, **120**, 549-558, (1970).
- 8 Laemmli, U. K., *Nature*, **227**, 680-685 (1970).
- 9 Kerr, I. M., Olshesky, U., Lodish, H. F., and Baltimore, D., *J. Virol.*, **18**, 627-635 (1976).
- 10 Smith, R. E., Zweerink, H. J., and Joklik, W. K., *Virology*, **39**, 791-810 (1969).
- 11 Waddell, W. J., *J. lab. clin. Med.*, **48**, 311-314 (1956).
- 12 Friedman, R. M., Metz, D. H., Esteban, M., Tovell, D. R., Ball, L. A., and Kerr, I. M., *J. Virol.*, **10**, 1184-1193 (1972).
- 13 Rubin, C. S., and Rosen, O. M., *A. Rev. Biochem.*, **44**, 831-887 (1975).
- 14 Lebleu, B., Sen, G. C., Shaila, S., Cabrera, B., and Lengyel, P., *Proc. natn. Acad. Sci. U.S.A.*, **73**, 3107-3111 (1976).
- 15 Zilberstein, A., Federman, P., Shulman, L., and Revel, M., *FEBS Letts*, **68**, 119-124 (1976).

Primary and secondary variants in immunoglobulin heavy chain production

WE have isolated numerous variants of a mouse myeloma cell line which synthesised altered heavy immunoglobulin chains and normal light chains after treating the line with the acridine mustard ICR-191 (refs 1-4). The four types of primary variants that we found are shown in Table 1. The primary variant clones were generally stable but we did find one clone which on recloning produced subclones synthesising a variant heavy chain with molecular weight lower than that synthesised by the primary clone. This observation raised the question of whether certain primary variants might have a built-in instability and that the original mutation might be affecting a regulatory process. We were thus led to seek additional examples of spontaneous secondary variant production to see if this were a more general phenomenon. Here we report our results.

In the earlier experiments we isolated many independent

clones which fell into the four primary variant types. Two types synthesised heavy chains which are shorter than the parent and which lack the parental subclass serotype; two types synthesise heavy chains of a different subclass serotype from the parent. We also characterised the variants by the manner in which they assemble their heavy and light chains and by peptide analysis¹⁻⁴. Occasionally a primary variant clone showed decreased heavy chain synthesis while continuing to make the altered heavy chain. To retrieve those cells continuing to make the altered heavy chain, we recloned the primary variant cells. As expected, most subclones were of either the primary variant heavy chain type or the light chain producer type. The exception was the variant mentioned above synthesising a heavy chain of molecular weight 50,000: on recloning it we found, in addition to clones synthesising heavy chains of molecular weight 50,000 and clones that had lost the variant heavy chain synthesis completely, numerous subclones synthesising a variant heavy chain of molecular weight 40,000 (ref. 3).

In the present experiments the parental cell lines 45.6, 45.6.2.4 and 45.6.3.2 were derived from the MPC 11 tumour which synthesises an IgG-2b immunoglobulin⁵. The original variants were isolated from the parental population after mutagenesis with ICR-191^{3,4}. Mutagenised cells were cloned in soft agar and overlaid with an antiserum directed against either the completely reduced and alkylated MPC 11 (γ 2b) heavy chain or the Fc region (C-terminal half of the MPC 11 heavy chain). An antibody-antigen precipitate developed over those clones synthesising and secreting parental heavy chains. "Unstained" variant clones were retrieved from the agar and grown to mass culture. Unstained clones included clones which had ceased heavy chain synthesis completely, clones which continued heavy chain synthesis but had difficulty in secreting the heavy chain, and clones which synthesised an altered heavy chain not recognised by the overlay antiserum. The details of cell culture and cloning and the analysis of variant clones have been described previously^{3,4}.

To select secondary variants from individual primary variants synthesising altered heavy chains, we followed the same cloning-antibody overlay procedure without subjecting the cells to any additional mutagenesis. In this type of selection, the primary variant clones were "unstained" (as originally selected). "Stained" clones included those clones which secreted the primary variant heavy chain in sufficient quantity to yield a visible precipitate and those clones which synthesised and secreted a new immunoglobulin heavy chain which was recognised by the overlay antiserum. The same protocol would have selected clones synthesising and secreting the parental MPC 11 immunoglobulin, that is, revertants. To date, in several independent experiments, we have found no revertants.

Secondary variants have however been found in several experiments. In one study, a primary variant (45.6.3.2 ICR 11.19)^{3,6} which synthesised a heavy chain of molecular weight 50,000, was recloned and overlaid with an antiserum directed against the completely reduced and alkylated heavy chain of the MPC 11 immunoglobulin (IgG-2b). The primary variant had been selected originally as "unstained" by this same method. When the variant was recloned, sixteen of 478 clones were now found to be stained. Seven of these sixteen clones were grown to mass culture and analysed by Ouchterlony analysis of cytoplasmic contents and secreted immunoglobulin, and by electrophoresis of radiolabelled immunoglobulin on acrylamide gels containing sodium dodecyl sulphate. Five of the seven were identical to the primary variant (45.6.3.2 ICR 11.19) in that they synthesised heavy chains of molecular weight 50,000 which assembled with light chains to yield HL, L2 and L components. Now, however, they secreted the variant heavy chain in sufficient quantity to be detected as stained in the overlay procedure. The remaining two clones were found to synthesise heavy

Table 1 Size and serological characteristics of the heavy chains synthesised by variant types of the MPC cell line

Parent Variants	MPC 11	Size of heavy chain	Subclass	Anti:	Cytoplasm*			Anti:	Secretion*		
					H†	Fc‡	γ2a§		H†	Fc‡	γ2a§
	MPC 11	55,000	γ2b		+	+	—		+	+	—
	Short heavy chain	(1) 50,000	neg		+	—	—		—	—	—
		(2) 40,000	neg		+	—	—		+	—	—
	γ2a serotype	(3) 75,000	γ2a		+	+	+		—	—	—
		(4) 55,000	γ2a		+	+	+		+	+	+

*Ouchterlony analysis as in ref. 3.

†Anti-H = rabbit anti-MPC 11 heavy chains

‡Anti-Fc = rabbit anti-MPC 11 Fc.

§Anti-γ2a = rabbit anti-LPC1 (IgG-2a κ).

chains of increased (normal) size (molecular weight 55,000), which bore the γ2a serotype. Figure 1 shows the result of this experiment. This result is contrasted to the earlier experiment in which another independent clone of the same primary type 45.6 ICR 4.68 (ref. 3) gave rise, on recloning, to clones synthesising heavy chains of molecular weight 40,000. These experiments are summarised in Table 2.

In the second series of experiments, the single primary variant which had been previously characterised and shown to synthesise a γ2a heavy chain of molecular weight 75,000 (45.6.2.4 ICR 9) was recloned and overlaid with an antiserum directed against the Fc region of MPC 11 (γ2b) or with an antiserum directed against a γ2a immunoglobulin (LPC-1). Neither antiserum is specific for subclass determinants. Each is reactive with both γ2a and γ2b myeloma proteins. In the initial experiment, one of 660 clones was stained with the anti-MPC 11 Fc serum. This clone was found to be synthesising and secreting heavy chains which continued to bear the γ2a serotype, but were now of decreased (normal) size (molecular weight 55,000). The results are shown in Fig. 1.

In a subsequent experiment with 45.6.2.4 ICR 9, one of 766 clones was stained with the anti-γ2a antiserum. This stained clone continued to synthesise the primary variant heavy chain of molecular weight 75,000. Upon recloning this stained clone, 994 out of 995 clones were unstained, indicating a considerable degree of clonal variability. One of the 995 clones was stained and was now found to be making a γ2a chain of molecular weight 55,000.

In another independent experiment utilising anti-γ2a antiserum, five out of 1,150 clones were stained. Four of the five were identical to the primary variant except that they now secreted the variant heavy chain in sufficient quantity to be detected as stained in the overlay procedure. The remaining clone synthesised a heavy chain of decreased (normal) size (molecular weight 55,000) having the γ2a serotype. This secondary variant was quite stable, since on recloning, all of 3,000 subclones were stained with the anti-γ2a antiserum and 15 subclones examined by radiolabelling and electrophoresis on acrylamide gels were identical in the size and assembly patterns of the heavy and light chains they synthesised.

The primary variants which gave rise to secondary variants have certain features in common. Neither the molecular weight 50,000 heavy chain nor the molecular weight 75,000 heavy chain is assembled well into H₂L₂ and

neither is secreted well from the cell. The assembly patterns of these primary variants are shown in Fig. 1. Note the relative lack of H₂L₂ in panels a1 and b1 as compared with the relative abundance of H₂L₂ in panels a2 and b2. The

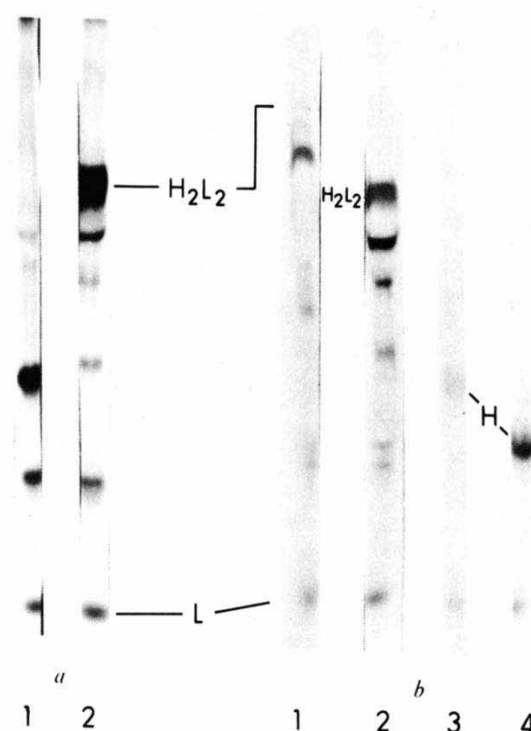


Fig. 1 Comparison by electrophoresis on sodium dodecyl sulphate-acrylamide gels of assembly patterns of primary and secondary variants. a1, 45.6.3.2 ICR 11.19, which synthesises a heavy chain of molecular weight 50,000, and the secondary variant, a2, 45.6.3.2 ICR 11.19 S3, which synthesises a γ2a heavy chain of molecular weight 55,000. b1, 45.6.2.4 ICR 9, which synthesises a γ2a heavy chain of molecular weight 75,000 and the secondary variant, b2, 45.6.2.4 ICR 9.9.2, which synthesises a γ2a heavy chain of molecular weight 55,000. Panels b3 and b4 show the respective heavy and light chains after total reduction and alkylation of the disulphide bridges. The assignment of H₂L₂, H and L is done in accordance with ref. 6 and confirmed by comparative molecular weights from these and other data.

Table 2 Conversion of primary variants to secondary variants

Size and serotype of parent chain	Parent cell line	Mutagen	Primary variant: name and size of heavy chain	Secondary variant: size of heavy chain	Serotype
IgG-2b (55,000)	45.6	ICR-191	(45.6 ICR 4.68) 50,000	40,000	Negative
	45.6.3.2	ICR-191	(45.6.3.2 ICR 11) 50,000	55,000	(γ2a)
	45.6.2.4	ICR-191	(45.6.2.4 ICR 9) 75,000	55,000	(γ2a)

diminished ability of some variants (45.6 ICR 4.68, 45.6.3.2 ICR 11.19, and 45.6.2.4 ICR 9) to secrete immunoglobulin heavy chains may confer a selective disadvantage on these cells, thereby allowing us to detect secondary variants in these populations. In contrast, primary variants synthesising heavy chains of molecular weight 40,000 and 55,000 seem quite stable. In the latter case, the heavy chains are assembled well into H_2L_2 (as shown in Fig. 1, panels a2 and b2) and are subsequently secreted from the cell^{3,4}. Consequently, both primary and secondary variants of these stable types are especially valuable since they secrete myeloma protein into the sera of mice bearing tumours induced by subcutaneous or intraperitoneal injection of the variant cells. Thus, structural studies of the variant heavy chains are facilitated.

Many observations suggest that all our variants are the result of mutation: the variants are generally stable and are increased in number after mutagenesis, and the heavy chains they synthesise differ structurally from the parent. In addition, the variants we have seen, whether primary or secondary in origin, seem to fall qualitatively into the four groups shown in Table 1. These groups are defined by size of the heavy chain, assembly characteristics, and serological characteristics even though the members of each group differ somewhat by peptide mapping and, in some cases, by rate of assembly. Perhaps the quantal characteristics of the sizes of variant heavy chains produced will reflect the presence of certain genetic hot spots which are sites of rapid mutation.

On the other hand, the primary variants arise at a remarkably high incidence. For example, treatment of the parental population with $1 \mu\text{g ml}^{-1}$ of ICR-191 yields unstained clones at an incidence of 2%. One-third of these clones are synthesising altered heavy immunoglobulin chains. These incidences are extraordinary when compared to measurements in bacteria and other mammalian cell culture systems. This observation, together with the spontaneous generation of secondary variants from certain primary variants, makes us question whether the original lesion is within the structural gene dictating the parental heavy chain. It is possible, for example, that exposure to certain mutagenic agents will render a cell generally hypermutable because of an interaction with a regulatory element. It is interesting, therefore, that so far variants synthesising heavy chains of molecular weight 50,000 or 75,000, that is, those which have subsequently yielded secondary variants, have been selected only after treatment with ICR-191. The two stable types, those which synthesise short heavy chains of molecular weight 40,000 and those which synthesise $\gamma 2a$ heavy chains of molecular weight 55,000 have appeared after treatment with either ICR-191 or with Melphalan^{4,7}, a phenylalanine mustard used in clinical treatment of human myeloma. Whether particular primary variants may have an essential instability is a question we are actively exploring.

We hope that the combination of cellular studies with the careful structural analysis of variant heavy chains will help us to analyse the genetic and molecular nature of the variations observed in these cells.

We thank Mr Richard Campbell for technical help. This work was supported by a grant from the NIH.

SAIJA KOSKIMIES*

BARBARA K. BIRSSTEIN

Department of Cell Biology,
Albert Einstein College of Medicine,
1300 Morris Park Avenue,
Bronx, New York 10461

Received August 13; accepted October 4, 1976.

* Present address: Department of Serology and Bacteriology, University of Helsinki, Haartmanink 3, 00290 Helsinki, 29 Finland.

¹ Bauml, R., Birshtein, B. K., Coffino, P., and Scharff, M. D., *Science*, **182**, 164-166 (1973).

- ² Birshtein, B. K., Preud'homme, J.-L., and Scharff, M. D., in *The Immune System Genes, Receptors, Signals: Third ICN-UCLA Symposium on Molecular Biology* (edit. by Sercarz, E. E., Williamson, A. R., and Fox, C. F.), 339-351 (Academic, New York, 1976).
- ³ Birshtein, B. K., Preud'homme, J.-L., and Scharff, M. D., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 3478-3482 (1974).
- ⁴ Preud'homme, J.-L., Birshtein, B. K., and Scharff, M. D., *Proc. natn. Acad. Sci. U.S.A.*, **72**, 1427-1430 (1975).
- ⁵ Laskov, R., and Scharff, M. D., *J. exp. Med.*, **131**, 515-541 (1970).
- ⁶ Weitzman, S., and Scharff, M. D., *J. molec. Biol.*, **102**, 237-252 (1976).
- ⁷ Preud'homme, J.-L., Buxbaum, J., and Scharff, M. D., *Nature*, **245**, 320-322 (1973).

Annihilation of positrons in D and L isomers of various amino acids

GARAY *et al.*¹ have reported on experiments on positron annihilation in D and L isomers of various amino acids. I show here that one of the two interpretations which they put forward to explain their results is in error, and also discuss other possible experiments which may enable clarification of the rather unusual results they have reported.

In the experiment referred to (paraphrasing the authors' description) 'crystalline samples of D and L amino acids were packed around a 0.8- μCi Na²² (positron) source to a thickness of 6-7 mm, and it (source plus amino acid) was placed between two scintillation counters of NE111 plastic scintillator coupled to 56-AVP photomultipliers'. The positrons either annihilate directly, probability > 80% and lifetime $\tau \sim 0.2$ ns, or form singlet or triplet positronium (Ps), with lifetimes $\tau_s \sim 0.1$ ns and $\tau_t \sim 1$ ns (τ_t is reduced from its free space value of 138 ns by electron pickoff). Using standard fast coincidence techniques the authors find the intensity of the long-lived (presumably triplet) Ps components in D and L isomers of a series of 5 amino acids to satisfy the relation $I_L/I_D = 0.67$ (7), 0.72 (6), 0.82 (5), 0.83 (6), 0.94 (6). The numbers in parentheses are experimental errors and the results of 0.67 and 0.82 come from samples of tryptophan produced by different laboratories. The authors conclude from this data that "... D isomers of amino acids favour triplet states in the case of forward polarised β^+ particles. It seems likely from this evidence that β decay was the cause of an initial asymmetry in the racemic mixtures present on the primordial Earth".

The authors interpret these results in terms of a possible correlation between the electron velocity and the electron spin within the sample, $\langle v_e \sigma_e \rangle = K \delta_{e\beta}$ where K has opposite sign for L and D and $\langle \rangle$ refers to an ensemble average. They also assume that the probability of Ps formation depends on the relative velocity of electron and positron. If then the positrons have a non-zero helicity h ($h = \langle \sigma(e^+) \cdot V(e^+) \rangle$ where $\sigma(e^+)$ is the e^+ spin vector, $V(e^+)$ the e^+ velocity direction, and $\langle \rangle$ refers to an ensemble average over the e^+ beam) the probability of triplet against singlet Ps formation will vary in switching from L to D. Thus one would expect that the intensity of the short and long components in the time spectrum should depend on the isomer under consideration. Note that in this interpretation it is the helicity, not the polarisation $P = \langle \sigma(e^+) \rangle$ of the beam, on which I_L/I_D depends. As an instructive example to illustrate this point consider the limiting (and completely artificial) cases in which Ps formation occurs only for $V(e^-)$ parallel to $V(e^+)$ and for $K = +1$ for L and -1 for D. If we now note that the spinor direct products $\uparrow \uparrow$ or $\downarrow \downarrow$ ($\uparrow, \downarrow; \uparrow, \downarrow$ refer to e^-, e^+ spins) give half triplet and half singlet decays one may easily show that the situation $P=0$ for $h = +1-1$ yields $I_L/I_D = 2$ or 0.5 while $h = 0$ yields $I_L/I_D = 1$ for any P .

We now remark that whatever the correlation between Ps formation and relative velocity and whatever the actual (presumably small) value of K is, $h \approx 0$ at e^+ kinetic energies ($\sim 5 \text{ eV} - 15 \text{ eV}$) characteristic of Ps formation. This can be demonstrated by noting that experimental² and theoretical^{3,4} studies have shown that if a beam of electrons (or presumably positrons) impinges on a surface with energy E_0 ($10^4 \text{ eV} < E_0 < 10^6 \text{ eV}$) the beam will have a completely diffuse nature (velocities

completely random) by the time it has penetrated a distance where the mean energy of its constituent particles is still $> 3,000$ eV. This may be most readily arrived at by applying formulas (4) and (5) of Archard⁴ for the case $Z \sim 3-10$ (the result is insensitive to Z). Since at energies $\leq m_0c^2$ ($=0.5$ MeV) σ does not follow V (see ref. 5) it follows that $h \simeq 0$. Note that the energy range 10^4-10^6 eV brackets the energy of almost all e^+ emitted by ^{22}Na . In addition, though it is not relevant to the problem, we point out that the geometry of the experiment gives $P \simeq 0$ as well.

We conclude that the effect seen by Garay *et al.* is in all likelihood not a result of positron polarisation or helicity. If real it may be a result, as Garay *et al.* recognise, of the inhibition of Ps formation and/or greater triplet quenching in L as opposed to D, possibly because of an undetected chemical impurity. It may also be caused by some slight difference between the solid state structure of the L and D crystals. If so, a new simple, non-destructive and highly sensitive method of detecting such impurities is now available. Experiments to determine the dependence (or lack thereof) of I_L/I_D and the overall Ps formation rate on P and possibly on h are now being started. To try to investigate the dependence of I_L/I_D on h we may use the low energy positron beam available in our laboratory, if the polarisation of the beam is sufficiently high.

I thank G. W. Ford, D. W. Gidley, A. Nudelfunden and E. Rosenberg for useful conversations, and the NSF for support.

ARTHUR RICH

Department of Physics,
The University of Michigan,
Ann Arbor, Michigan 48109

Received June 29; accepted September 29, 1976.

¹ Garay, A. S., Keszthelyi, L., Demeter, I., and Hrasco, P., *Nature*, **250**, 332-333 (1974); *Chem. Phys. Lett.*, **23**, 549-552 (1973).

² Cosslett, V. E., and Thomas, R. N., *Br. J. appl. Phys.*, **15**, 883-907 (1964).

³ Bethe, H. A., Rose, M. E., and Smith, L. P., *Proc. Am. phil. Soc.*, **78**, 573-585 (1938).

⁴ Archard, G. D., *J. appl. Phys.*, **32**, 1505-1509 (1961).

⁵ Bouchiat, C., and Levy-Leblond, J. M., *Nuovo Cim.*, **XXXIII**, 193-200 (1964).

Conformations of double-helical nucleic acids

A KNOWLEDGE of possible conformational states of double-helical nucleic acids is fundamental to an understanding of how they work^{1,2}. Models for a series of conformations have been made on the basis of X-ray data³⁻⁵. Investigations of nucleic acids in solution point to the existence of a continuum of forms but do not make possible the quantitative determination of the conformational parameters. Here, theoretical investigation of the intramolecular interaction energy as a function of conformational parameters and a search for minimal energy regions in the space of these parameters become essential.

Two families of nucleic acid conformations are known, the A and B families, which differ by the mutual position of the base pairs, the conformational angles in the ribose-phosphate backbone and the conformation of the ribose ring. This last is a major factor determining whether a double-helical structure belongs to the A or B family of conformations. The C_3' -endo conformation in the A family and the C_2' -endo or C_3' -exo conformation in the B family are assumed⁴. Data on the conformation of the ribose ring cannot be obtained from the diffraction patterns of nucleic acid fibres. The ribose conformation is taken from X-ray data for the nucleic acid components. The ribose ring in double-stranded helical nucleic acids is, however, in different surroundings from that in monomer crystals. It may be rearranged in the course of transitions between both the families and the different forms of a family. In this connection, the intramolecular interaction energy of double-helical nucleic acids was considered in our work (unlike the recent

works^{6,7}) with an account of conformational possibilities of a ribose ring.

We calculated the intramolecular interaction energy for the regular double-helical polynucleotide containing rigid Watson-Crick pairs as a function of eight conformational variables. Four of them determine the mutual position of the base pairs (D is the distance from the pair centre to the helical axis, d the pair-pair distance along the helix axis, θ the tilt angle of the base pairs to the plane perpendicular to the helix axis, τ the rotation angle of one pair with respect to another around the helix axis), four others ($OC_1'C_2'$ and $C_1'C_2'C_3'$ bond angles and dihedral angles τ_1 and τ_2) determine the conformation of a ribose ring. The bond angles of a ribose ring were considered as variables in contrast with other bond angles. The bond lengths were fixed. The eight variables with the above-mentioned

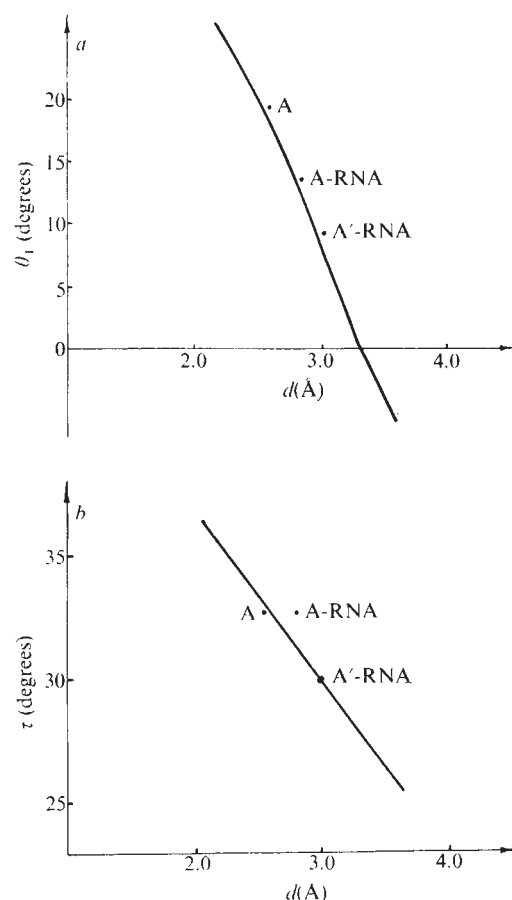


Fig. 2 Projections of the B-family bottom position for the poly(dA).poly(dU): a, on the (D , θ); and b, on the (D , τ) planes. The known forms are marked.

constraints completely determine the conformation of a regular polynucleotide.

Dihedral angles of sugar-phosphate backbone were determined by means of a procedure which resembles one of Go and Scheraga's⁶. The van der Waals' interactions were calculated by the atom-atom potential function method⁹. The Lennard-Jones potentials were used; the validity of parameters was verified by calculations on nitrogen base crystals. The heats of sublimation and lattice constants of a number of aromatic compound crystals were reproduced (V.I.P., unpublished). The tension energy of the bond angles in the ribose ring was assumed to be proportional to the square of the deviation of these angles from the tetrahedral ones. Previously¹⁰ we studied the interaction energy between the base pairs as a function of parameters determining their mutual position in a double helix. We detected a limited region in the space of these parameters

energetically allowable with these parameters. Thus the region of the eight-dimensional conformational space which should be investigated might be drastically diminished. The many-variable function minimisation procedure was used for searching for the energy minima.

The calculations showed the existence of two valley-like regions of minimal values on the energetic surface. One of them corresponds to the A family of nucleic acids, the other to the B family. The energy variation along the bottom of each valley is rather small; the energies are the same for both valleys up to 1 kcalorie mol⁻¹. The points which correspond to the models constructed by means of X-ray data are placed in the conformational space near the lines which describe the position of the bottom of a valley. There is a systematic deviation towards the lower D values for the points which correspond to the models of the A forms. But if $D = 4.7$ Å is fixed (like the models of the A forms) the line in the conformational space which corresponds to the bottom of this valley passes through the experimentally determined forms of the A family. This displacement is connected with the intramolecular interaction energy change by 1–2 kcalorie mol⁻¹. It is not excluded that the double-helical macromolecule contracted in diameter due to intermolecular interactions with neighbouring molecules in fibres. The A family is characterised by a nearly linear relationship between the parameters d and θ as well as d and τ (Fig. 1). The forms both with great and with small distances between the pairs along the helix axis are sterically allowed. The position of the valley bottom corresponding to the B family is characterised by the constancy parameter d (approximately 3.4 Å). For the B family, τ increases almost linearly whereas θ strongly diminished with the decrease of D in accordance with the

Fig. 1 Projections of the A-family bottom position for the poly(dA).poly(dU): a, on the (d , θ); and b, on the (d , τ) planes ($D = 4.7$ Å). The known forms are marked.

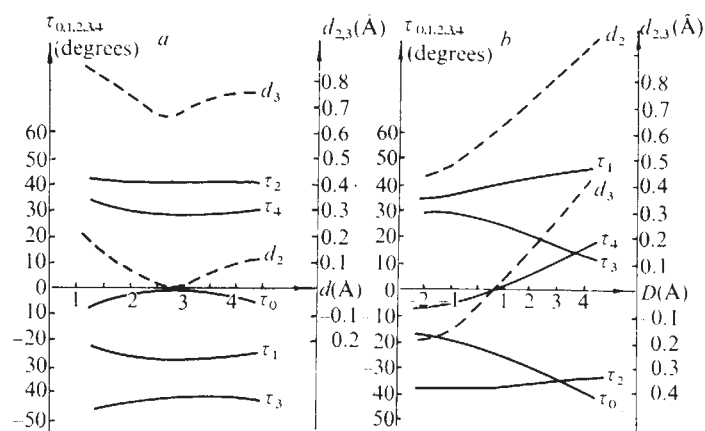
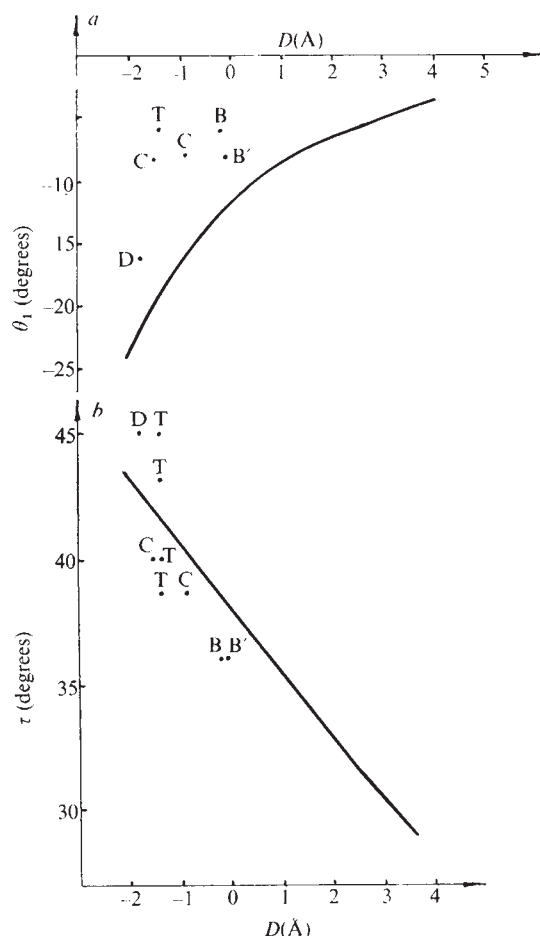


Fig. 3 Dependence between the changes in the conformational parameters of deoxyribose ring and the position of the structure on the valley bottom for A (a) and B (b) families. $\tau_0, \tau_1, \tau_2, \tau_3, \tau_4$: rotation angles around $O'-C_1', C_1'-C_2', C_2'-C_3', C_3'-C_4', C_4'-O'$ respectively; d_2 and d_3 : the derivations of the C_2' and C_3' atoms from the plane of other atoms of a sugar ring. The positive values corresponds to deviation towards C_5' atom.

results of X-ray studies (Fig. 2). A systematic deviation towards the lower θ values can be seen for the points which correspond to the models of B family forms but there is a tendency to raise absolute value of θ in recent models.

A correlation is observed between the disposition of the base pairs which corresponds to the optimal energy values and the sugar ring conformation (Fig. 3). The last one resembles the standard C_2' -endo or C_3' -endo conformation for those parts of valleys where models based on X-ray studies exist. The ring deformation in the B family is more pronounced than that in the A family. At the positive D values both C_2' and C_3' atoms deviate from the $C_1'O'C_4'$ plane towards C_5' (Fig. 3b).

An energetically unfavourable region exists in the conformational space between the A and B family valleys. The minimal steric barrier between the two families is about 3 kcalorie mol⁻¹.

The relationships between the conformational parameters of a double-helical polynucleotide which we have obtained may be used to detect the degrees of freedom of the system for the analysis of its behaviour under the effect of weak perturbations (as against intramolecular interactions). Different double-helical nucleic acid conformations may exist in different conditions in the interaction with some biologically important compounds.

We thank the head of our laboratory A. I. Kitaigorodskii for his interest and discussions.

V. E. KHUTORSKY
V. I. POLTEV

Institute of Biological Physics,
Academy of Sciences of USSR,
Pushchino, Moscow Region, 142292 USSR

Received June 15; accepted September 27, 1976.

1. Arnott, S., Fuller, W., Hodson, A., and Prutton, I., *Nature*, **220**, 561–564 (1968).
2. Florentiev, V. L., and Ivanov, V. I., *Nature*, **228**, 519–522 (1970).
3. Arnott, S., *Progr. Biophys. mol. Biol.*, **21**, 265–319 (1970).
4. Arnott, S., and Selsing, E., *J. molec. Biol.*, **98**, 265–269 (1975).
5. Mokulsky, M. A., Kapitonova, K. A., and Mokulskaya, T. D., *Mol. Biol.*, **6**, 883–901 (1972).
6. Zhurkin, V. B., Lysov, Yu. P., and Ivanov, V. I., *FEBS Lett.*, **59**, 44–47 (1975).
7. Calascibetta, F. G., Dentini, M., De Santis, P., and Marosetti, S., *Biopolymers*, **14**, 1667–1684 (1975).
8. Go, H., and Scheraga, H. A., *Macromolecules*, **3**, 178–187 (1970).
9. Kitaigorodskii, A. I., *Molecular Crystals and Molecules* (Academic, New York, 1973).
10. Khutorsky, V. E., and Poltev, V. I., *Molec. Biologia*, **9**, 747–751 (1975).

matters arising

Particle creation and Dirac's Large Number Hypothesis

IN a recent paper¹ Steigman claims to show that the creation of matter as postulated by Dirac² is unnecessary. In particular he claims that a theory with a gravitational constant G varying as t^{-1} automatically implies that the number of nucleons within the horizon should increase as t^2 . If so, Dirac's hypothesis that $N \sim t^2$ must be due to spontaneous matter creation becomes unnecessary. We here show Steigman's claim to be incorrect.

First, note that within Dirac cosmology the present radius of the Universe varies as $R \sim t$. Assuming such a time dependence holds in the very early Universe, as Steigman does, then there is no horizon since the integral

$$d_H(t) \equiv cR(t) \int_0^t \frac{d\tau}{R(\tau)} \quad (1)$$

defining the horizon distance diverges logarithmically. Therefore, any increase of mass, M , with time, t , no matter how, must be ascribed to spontaneous creation since no matter is entering the space, V , across the expanding horizon.

We now show how matter creation must be an independent hypothesis for Steigman's equation (1) to be correct. As shown in ref. 3, the correct Einstein equation in atomic units is given by

$$\left[\frac{\dot{R}}{R} + \frac{\dot{\beta}}{\beta} \right]^2 + \frac{k}{R^2} = \frac{8\pi}{3} \bar{G} \bar{\rho} \beta^2 \quad (2)$$

Here β is the gauge function which varies asymptotically as t^{-1} , and $\bar{G}$ and $\bar{\rho}$ are given in Einstein units. In Einstein units $\bar{G}$ is constant, and $\bar{\rho}$ is given by

$$\bar{\rho} = \bar{M} / \bar{R}^3 \quad (3)$$

where, following Dirac², $\bar{M}$ and $\bar{R}$ are constant so $\bar{\rho}$ is constant. Since $\bar{R} = \beta R$, where R and R are the radii of the Universe in Einstein and atomic units, respectively, equation (2) becomes

$$G\bar{\rho}\beta t^2 = \text{constant} \quad (4)$$

where we have used $G = \bar{G}\beta \sim t^{-1}$ and

$R(t) \sim t$. Equation (4) coincides with Steigman's equation (1) only if we define

$$M/R^3 \equiv \rho \equiv \bar{\rho}\beta = \bar{M}\beta/\bar{R}^3 \quad (5)$$

Since $\bar{R} = \beta R$ it follows that the mass entering the definition of ρ , that is, M , must be related to the constant mass $\bar{M}$ by the relationship

$$M = \bar{M}/\beta^2 = \bar{M}t^2 \quad (6)$$

which explicitly shows matter creation.

The error in Steigman's reasoning lies in the postulate of his equation (1) which does not have the backing and theoretical framework that justifies and correctly defines each quantity in the equation. One cannot simply postulate the existence of a relationship of the type

$$G\rho t^2 = \text{constant} \quad (7)$$

and then take $G \sim t^{-1}$ assuming, as Steigman does, that ρ does not contain matter creation. If such a theory exists, as it might well do, it cannot be presented under the name Dirac cosmology. In fact, the full Dirac theory yields a relationship like equation (7) only if ρ is defined by equation (5), that is, if it already contains matter creation.

The second part of Steigman's criticism is invalid for a different reason. Here he points out that Dirac's large Number Hypothesis (LNH) implies such things as the fact that there can be no primordial nucleosynthesis, and that for $T \sim 10^{10}$ K the number of baryons in the Universe is of order unity.

In fact Dirac² has repeatedly stressed that the LNH is an asymptotic theory valid only for relatively large times over most of the age of the Universe, but not necessarily at very early epochs. Thus the second part of Steigman's paper strongly supports Dirac's statements and in no way invalidates the LNH.

In summary, Steigman's claim that Dirac's LNH does not require particle creation is wrong because he has assumed that which he was seeking to prove, that is that ρ does not contain matter creation. Steigman's claim that Dirac's LNH leads to nonsensical results in the very early Universe is superficially correct, but this only supports Dirac's contention that the LNH may not be valid in the very early Universe.

V. CANUTO*

P. J. ADAMS

S. H. HSIEH

E. TSIANG

Goddard Institute for Space Studies,
NASA, New York, 10025

*Also at the Department of Physics, City College,
CUNY, New York, New York, 10031

¹ Steigman, G., *Nature*, **261**, 479 (1976).

² Dirac, P. A. M., *Proc. R. Soc.*, **A338**, 439 (1974).

³ Adams, P. J., Canuto, V., Hsieh, S. H. and E. Tsiang, *Phys. Rev. D*, (in the press).

STEIGMAN REPLIES—In Dirac's original cosmology the scale factor varies as $R \sim t^{1/3}$ (there is a possible confusion by Canuto *et al.* between 'scale factor' and 'radius of the Universe'). In this model there is a horizon and the results and conclusions of my paper apply. There are, of course, a large number of possible modifications of Dirac's original model. In the variation chosen by Canuto *et al.*¹ ($R \sim t$) there is no horizon. In this model then, there are always an infinite number of particles 'in the Universe' and, clearly, Dirac's Large Number Hypothesis (LNH) cannot apply.

Canuto *et al.* argue that several of my criticisms of Dirac's theory are invalid because the LNH is an 'asymptotic' theory. It is certainly not clear why this should be so; why do the equations in Canuto *et al.* not apply for all times? A cosmological theory which only predicts the present epoch is of questionable value.

National Radio Astronomy Observatory
Charlottesville, Virginia 22901

¹ Canuto, V., Adams, P. J., Hsieh, S. H., and Tsiang, E., *Nature*, **263**, 485 (1976).

The pre-palaeozoic basement in south-eastern Scotland and the southern uplands fault

BASAMENT xenoliths in Carboniferous volcanics in the vicinity of Partan Crag along the south side of the Firth of Forth have been used to infer the character of the immediately underlying basement by Upton *et al.*¹. These

xenoliths are probably representative of a subjacent basement but they cannot be unequivocally regarded as reflecting the basement character beneath the geological Midland Valley of Scotland, in a larger structural sense, as has been argued. Although the south-east boundary of the Midland Valley, as a geological feature, has been commonly taken along the Lammamuir Fault², it is likely that this fault may only be a shallow and relatively insignificant feature at depth. The magnetic map³ shows that the major geophysical break or linear, which, to the west, follows the course of the Southern Uplands Fault, passes near to or north of Partan Crag. The prominent magnetic linear lies everywhere between the Southern Uplands Fault and the faults associated with the structural hinge that passes from near Girvan to Edinburgh⁴. This suggests that either or both of these structures on the surface reflect the true position at depth of a significant crustal structure, which should be termed the Southern Uplands Line. It is suggested that the use of the Southern Uplands Fault alone as a single line taken as marking the surface expression of a major deep seated structure is unrealistic.

Along the south-east part of the Midland Valley, the magnetic linear³, which along the south-west margin is nearly coincident with the Southern Uplands Fault, diverges to the north from the geological southern margin of the Midland Valley in the vicinity of Dalkieth and passes near the southern margin of the Firth of Forth. There is a north-south distance of up to 18 km between the Southern Uplands Fault to the south, and the southern margin of the structurally more significant magnetic linear. The broad, open pattern of extensive, regular magnetic signature with shallow gradients, which can be traced through the Southern Uplands³, clearly extends beneath and North of the Lammamuir (Southern Uplands?) Fault and strongly suggests that rocks which are magnetically characteristic of those below the Southern Uplands pass northwards to at least the magnetic linear zone. This recognition of the probable northwards extent of the Southern Uplands basement would place the Southern Uplands basement very near to Partan Crag. It could then be argued that the basement rock types noted by Upton *et al.*¹, probably are characteristic of the basement beneath the Southern Uplands. This would be consistent with the basement character suggested from the western end of the Longford-Down Massif in Ireland⁵, which is a geophysical and probable geological extension of the Southern Uplands^{6,7}.

As it is uncertain whether the in-

ferred pre-Dalradian basement of Upton *et al.*¹ underlies the Midland Valley or the Southern Uplands tectonic zones, it would probably be unwise to be too specific about structural reconstructions involving the precise location of the landmass lying to the

Matters arising

Matters Arising is meant as a vehicle for comment and discussion about papers that appear in *Nature*. The originator of a Matters Arising contribution should initially send his manuscript to the author of the original paper and both parties should, wherever possible, agree on what is to be submitted. Neither contribution nor reply (if one is necessary) should be longer than 300 words and the briefest of replies, to the effect that a point is taken, should be considered.

south of the Dalradian sedimentary basin. The relationship of the xenoliths to the basement area they have been derived from must first be established with greater precision. If there is a significant difference between the basements beneath the Midland Valley and the Southern Uplands in SE Scotland, and this is not altogether certain, then the xenoliths could probably be better regarded as representing the basement to the south of the Southern Uplands line, rather than that to the north, beneath the Midland Valley.

MICHAEL D. MAX

Geological Survey of Ireland,
14 Hume St, Dublin 2, Ireland

- ¹ Upton, B. G. J., Aspen, P., and Graham, A., *Nature*, **260**, 517 (1976).
- ² Greig, D. C., *Inst. Geol. Sci.*, Pt. 13 (1971).
- ³ Butterwell, W., *Inst. Geol. Sci. Sheet 1* (1972).
- ⁴ George, T. N., in *The British Caledonian* (1963).
- ⁵ Strogen, P., *Nature*, **250**, 562 (1974).
- ⁶ Powell, D. W., *Scot. J. Geol.*, **6**, 353 (1970).
- ⁷ Murphy, T., *Inst. Coxs Phys. Dublin Bulls.*, **11** (1955).

Running Dinosaurs

ALEXANDER'S¹ use of stride length and footprint size to determine from trackways the speed of terrestrial vertebrates will greatly increase our understanding of dinosaurian behaviour. None of the trackways he cited were of large running dinosaurs, although he did not suggest that large dinosaurs never ran. We herein cite one such example, and another of a very fleet dinosaur that was not running.

In a trackway of a giant bipedal ornithischian from the Cretaceous (Campanian) of Colorado² the length of the footprint (*l*) is 0.86 m and the stride (λ) is 9.25 m. Similar footprints have been referred to the Hadrosauridae, the only large bipedal ornithischians then present in North America (ref. 3: footprint in collections of Drumheller and District Museum, Alberta). Combining Alexander's method to find hip height (*h*; $h=4l=3.44$ m) with Colbert's⁴ method for estimating the weight of an *Anatosaurus* specimen (where $h=2.25$ m), it is apparent that the Colorado animal weighed about 11 tonnes or as much as two bull African elephants combined. This exceeds by a factor of nearly three the weights obtained for hadrosaurids from the northern interior of North America⁵, but is less than what we estimate (13.8 t) for a giant lambeosaurine hadrosaurid from Mexico⁶. We obtained our estimate using Colbert's⁴ method and considering a 1/12 scale model of *Hypacrosaurus altispinus* (NMC 8501) with a volume of 1,372 ml as a 1/17.5 scale of the Mexican lambeosaur (based on humeral lengths in the two specimens). According to Alexander's formula, the Colorado animal was running at a velocity of 7.54 m s⁻¹ (27.1 km h⁻¹).

Anatomically, it has been suggested that some ornithomimids weighing about 140 kg could attain speeds of 80 km h⁻¹ (ref. 6). (A 1/6.5 scale model of the ornithomimid *Dromiceiomimus breviterius* (NMC 12228) has a volume of 564 ml, yielding a weight of 154.9 kg, using Colbert's⁴ method.) In an ornithomimid trackway from the Campanian of Alberta⁷ λ is 1.88 m and we estimate *h* to be 1.22 m, indicating a speed of 1.77 m s⁻¹ (6.4 km h⁻¹). According to Alexander¹, this animal would have begun to run when λ/h reached 2, or at a speed of 2.75 m s⁻¹ (9.9 km h⁻¹). At a speed of 80 km h⁻¹, prints from the same foot (λ) would be 8.6 m apart.

In our opinion, trackway data so far obtained are not yet adequate for generalisation on dinosaurian metabolism.

D. A. RUSSELL

P. BÉLAND

National Museum of Natural Sciences,
Ottawa, Canada

- ¹ Alexander, R. McN., *Nature*, **261**, 129-130 (1976).
- ² Brown, B., *Nat. Hist.*, **41**, 190-202, 235 (1938).
- ³ Langston, W., *Nat. Hist. Papers, Natl. Mus. Canada*, **4**, 1-9 (1960).
- ⁴ Colbert, E. H., *Nov. Am. Mus. Nat. Hist.*, **2076**, 1-16 (1962).
- ⁵ Morris, W. J., *J. Palaeont.*, **46**, 777-779 (1972).
- ⁶ Russell, D. A., *Can. J. Earth Sci.*, **9**, 375-402 (1972).
- ⁷ Sternberg, C. M., *Bull. Geol. Surv. Canada*, **44**, 85-87, 134-135 (1926).

reviews

Membrane-bound glycoproteins

Gilbert Ashwell

Membrane Glycoproteins: A Review of Structure and Function. By R. Colin Hughes. Pp. 367. (Butterworth: London and Boston, Massachusetts, March 1976.) £15.00.

FOR years, the presence of carbohydrates covalently linked to protein molecules was regarded with disbelief, dismay, or even indifference, by classical protein chemists intent on structural determination. Eventually, as a result of the brilliant pioneering studies of men such as Dische, Neuberger, and Winzler, delineation of the chemical bond between carbohydrates and proteins was accompanied by a growing awareness of the role of surface glycoproteins as 'informational macromolecules' mediating a variety of biological phenomena on a cellular level. As a consequence, the past decade has seen a logarithmic growth in the number of publications documenting the ubiquitous presence and complex interactions of membrane-bound glycoproteins.

It is to this problem that the author has addressed himself in a valiant and impressive effort to organise, systematise, and summarise the diffuse and multidisciplinary reports which comprise the current literature on glycoproteins. It is, perhaps, the latter consideration which epitomises the difficulty in maintaining an integrated perspective on the relevance of findings in widely diverse scientific disciplines. Few investigators, actively pursuing their immediate research interests, are either willing or able to devote the time and effort necessary to master the vast amount of potentially relevant information available in journals and disciplines with which they have an only peripheral acquaintance.

Fortunately, that task has been brilliantly accomplished in this monograph. Beginning with a detailed examination of the methodology used in the detection and distribution of membrane glycoproteins, a number of well referenced tables are provided to summarise the important properties and chemical composition of plasma membranes isolated from various species, the surface characteristics of the commonly used tissue culture lines, and the availability of specific glycosidases useful in the analysis of terminal carbohydrate residues. Although

the major thrust of current studies on cell surface glycoproteins has been directed mainly at the outer surface of the cell, the membranes of the subcellular organelles are similarly rich in glycoproteins, and their participation in intracellular phenomena is largely speculative. This problem is considered in some detail in a provocative chapter which summarises the available information and seeks to interpret current views on the genesis of primary and secondary lysosomes.

Following this, the role of oligosaccharide determinants involved in human blood group activity and the chemical nature of the ABO and MN antigens is reviewed. The judicious use of illustrative diagrams and chemical formulae facilitate comprehension of the necessarily cumbersome nomenclature used for carbohydrate chains. This section also provides a good overview of the chemistry of the histocompatibility antigens which is quite adequate for the non-specialist reader; extensive referencing is included for those seeking more detailed information. A minor point that requires comment is the statement (p126) that histocompatibility antigens are precipitated by anti-H antisera. To the best of this reviewer's knowledge this is not quite accurate in that a second precipitating agent is always required.

The middle portion of the book covers the extensive literature on lectins and their fascinating reactivity

toward the membrane glycoproteins of the lymphocyte, with emphasis on the biological implications of cellular transformation. Finally, the current status of the biosynthetic and degradative mechanisms involved in the overall metabolism of glycoproteins is presented in elaborate detail. Especially noteworthy is the section on lipid intermediates which is written with commendable lucidity and insight. The discussion on the role of specific carbohydrates in the 'recognition' phenomena is thoughtful and well-documented. The speculation (p297), however, that the hepatic binding protein participates as a glycosyltransferase in the adhesive properties of membranes, as described by Roseman, is unlikely since the purified binding protein was shown to be devoid of both sialyl- and galactosyl-transferase activity.

In summary, this monograph provides an impressive synthesis of the glycoprotein literature up to and including 1974. The author has earned the gratitude of innumerable investigators in diverse disciplines, who are concerned with the biochemical basis of membrane function and its role in cell biology.

Gilbert Ashwell is Chief, Laboratory of Biochemistry and Metabolism, US Public Health Service, National Institutes of Health, NIAMDD, Bethesda, Maryland.

Membrane recipes

Biochemical Analysis of Membranes. Edited by A. H. Maddy. Pp. ix+513. (Chapman and Hall: London; Halsted: New York, July 1976.) £16.50.

THIS is a most timely book. In recent years a considerable expertise has grown up in membrane biology. But all the expertise in the world is wasted unless studies are carried out using pure samples of known origin. For studies of molecular events in membranes, the problem boils down to the isolation and characterisation of single membrane fractions with minimal loss of intrinsic components or gain of extrinsic components. Although progress in this direction has been fairly rapid, the development of suitable procedures

has remained more of an art than a science: hence the need for a book of this kind.

What Dr Maddy has done is to persuade a number of authors to present background discussion and experimental recipes for the preparation and analysis of the most common membranes and membrane constituents. The general format of each chapter is excellent. The first part consists of a comparative literature survey and the second of fully detailed experimental procedures, aimed largely at the level of the novice. The only real test of a collection of recipes is, of course, to try them in the kitchen, and this I have not yet had a chance to do. The couple of procedures published here

with which I am familiar, however, include all the useful tricks, and the others have an authoritative look about them.

The first section of the book consists of procedures for the isolation of membranes, and includes chapters on Mycoplasma membranes (Razin and Rottem), surface membranes (Neville), mitochondrial membranes (Sottocasa), endoplasmic reticulum (De Pierre and Dallner) and nuclear membranes (Harris and Agutter). The only pity here is that there is nothing on membranes of bacteria such as *Escherichia coli* or on micro-organisms such as *Tetrahymena*, both of which are proving of importance in studying correlations between membrane structure and function.

The second section of the book is concerned more with the analysis of membranes, and includes chapters on the solubilisation of membranes (Maddy

and Dunn) and on the analysis of membrane proteins (Dunn and Maddy), lipids (Veerkamp and Broekhuysen) and carbohydrates (Cook). These are followed by some more peripheral, but no less interesting chapters on the applications of phospholipases (Zwaal and Roelofs), on immunochemical gel precipitation techniques (Bjerrum and Bog-Hansen) and on protein labelling (Hubbard and Cohn).

It is perhaps a sign of how far the study of membranes has progressed that a book of this kind can be written; and there can be few working with membranes who would not gain by browsing through it. Finally, there is a coherence about the book which is rare to find in an edited volume, and for which the editor deserves high praise.

A. G. Lee

Dr Lee is a lecturer in the Department of Physiology and Biochemistry at the University of Southampton, UK.

Transition metal compounds

Methodicum Chemicum. Volume 8: Preparation of Transition Metal Derivatives. Edited by Kurt Neidenzu and Hans Zimmer. Pp. x+579. (Academic: New York, San Francisco and London, 1976.) \$110; £67.10.

THIS text details published and proven methods for the preparation of transition metal compounds. In general, one chapter is devoted to each of the thirty d-transition elements; the lanthanide and actinide groups of elements each merit one chapter, a summary of ferrocene chemistry is included; and aspects of carbonyl, metallocene, and heteropolyanion chemistries are reviewed separately. The material for each chapter is classified in order of increasing oxidation state, a reasonably good index is provided and no real difficulty should be encountered in using the volume as a reference source. Each chapter is referenced in a reasonably comprehensive manner and nearly 7,000 original publications and review articles are cited in all. Some 29 authors have made one or more contributions, and, in several instances, these chemists are internationally recognised as authorities on their chosen topic.

Despite the considerable collective talent and effort involved in the preparation of this work, however, several reservations must be expressed when considering a recommendation to purchase this expensive volume. The most serious of these reservations results from the inherent nature of this work, which attempts to describe contemporary synthetic transition metal chemistry

in less than 600 pages. Since the space devoted to a particular element is no more than fifteen pages, the book reads like a laundry list. The description of the preparation of any particular compound, or group of compounds, is covered in one sentence of the 'add A to B to produce C' variety, with no details of the yield, purification procedure(s), necessary precautions, or properties of the compound(s) being given. As none of the material is original and the authors have been critical only in their selection of the material to be included, much of the information presented is readily accessible to the initiated inorganic chemist in standard and specialist texts and review articles. These criticisms are all the more serious since, due to certain unspecified difficulties, a considerable delay has occurred between the submission of the manuscripts and their publication, so that the coverage of the literature ends at 1970.

This volume is an extremely useful, if somewhat outdated, reference source for synthetic routes developed for the preparation of a wide range of transition metal compounds. In view of the reservations outlined above, the inorganic research chemist could spend the amount of money involved more wisely by purchasing a collection of other texts. The non-specialist inorganic chemist, and other scientists interested in the preparation of inorganic compounds, should, however, find this compilation of considerable value.

C. David Garner

Dr Garner is a Lecturer in Inorganic Chemistry at the University of Manchester, UK.

Electrophilic halogenation

Electrophilic Halogenation. By P. B. D. de la Mare. Pp. xi+231. (Cambridge University: Cambridge, 1976.) Hardcover £10.50; softcover £4.50.

ALTHOUGH some discussion of the importance and mechanism of electrophilic halogenation is to be found in most undergraduate texts, there is a need for an up-to-date, detailed and unified discussion of such reactions.

In this book electrophilic halogenation is covered from a mechanistic viewpoint. It is a complex area where the problems of reactivity, regioselectivity, stereoselectivity, product multiplicity and the nature of the halogenating species all have to be taken into account. The author, who has been an active research worker in this area for some thirty years, adopts an approach which considers the formation and fate of the carbonationic species produced by attack of electrophiles in general on unsaturated compounds, both aromatic and aliphatic. The basic patterns which emerge are then elaborated for each of the halogens and their derivatives in turn. Topics such as the acid and base catalysed α -halogenation of carbonyl compounds are covered in a chapter on miscellaneous electrophilic halogenation, and there is also a chapter on electrophilic halogenation of aromatic heterocycles. Introductory chapters provide the necessary background in physical organic chemistry and describe the halogens and their derivatives.

In keeping with the Cambridge University Texts philosophy, the level of the book is intended to suit final year undergraduate and early postgraduate students. The book contains many references to the original literature and is clearly written. It will nevertheless be demanding at the level intended, concentrating as it does on physical organic rather than descriptive aspects of the subject. The undergraduate may also feel that the area of chemistry covered for the price of the book is somewhat limited; and indeed the book may have had a wider appeal if the scope had been broadened to cover either electrophilic addition and substitution in general, or halogenation in general. The latter suggestion has much to commend it since free radical halogenation is also very important and frequently competes with the electrophilic process.

R. C. Storr

Dr Storr is a Lecturer in the Department of Organic Chemistry at the University of Liverpool, UK.

Protein polymerisation

Thermodynamics of the Polymerization of Protein. (Molecular Biology: An International Series of Monographs and Textbooks). By Fumio Oosawa and Sho Asakura. Pp. viii + 204. (Academic: London and New York, March 1976.) £6.80; \$16.75.

NATURE constructs the elementary blocks composing the constituents of living matter from small inanimate molecules which abound in our environment. The synthesis of the building blocks as well as of the highly specific functional macromolecules which they eventually form, is a complex process. It proceeds with the aid of elaborate machinery, specific templates and an ample supply of energy. Classical biochemistry and molecular biology study this elaborate process of creation and of disposal of biological macromolecules. It may have come as a surprise that spontaneous assembly of biological structures, unassisted by accessory means, could proceed as well. Indeed there exists an enormous variety of structures which form spontaneously from simpler protein constituents. For this process, which is often reversible and satisfies the laws of thermodynamics, Caspar and Klug have coined the term self-assembly.

The book by Oosawa and Asakura illustrates the impressive contribution of the Nagoya school to the problem of protein polymerisation. In addition to thermodynamics, kinetics, structural requirements, allosteric control, polymorphism and internal motility and flexibility of assembled structures are treated as well. Unfortunately, the bulk of the discussion is restricted to studies of actin and flagellin polymerisation from the authors' and other, not too distant, laboratories. About 76 entries in the authors' index are only perfunctorily listed in the list of references at the end of the volume. Although this represents a useful sectioned compilation, it does not provide the critical discussion (on varied approaches to an interesting problem) one expects from an authoritative monograph. For instance, on page 60 it is stated that in the trimer-disk transformation of TMV protein the enthalpy increases by about 77 kcalorie mol⁻¹, and another experiment gives 14–38 kcalorie mol⁻¹ for the same process. The possible causes of this discrepancy, deriving from the analysis of the dependence of equilibrium constants on temperature on the one hand and calorimetric measurements on the other hand, are not discussed.

In chapter 10, the reader not familiar with the subject may be misled with respect to the chronological development of the use of quasi-elastic light scattering in the study of the dynamics of macromolecular structures. I have doubts whether the reader to whom this monograph is addressed will easily follow the calculations in chapters 6 and 10, and appreciate their significance in terms of the experiments performed. The symbols in Table 3 on page 87 are intelligible to crystallographers and the statement on page 97—"Thermodynamic analysis of the polymerisation equilibrium of sickle cell hemoglobin is not enough to be compared with the above result"—is cryptic.

There are some printing errors, but not excessively many. The illustrations are clear and attractive. The cost is reasonable and should not prevent interested libraries as well as individuals from acquiring this compact, if somewhat one-sided, introduction to a topical field.

Henryk Eisenberg

Henryk Eisenberg is a member of the Polymer Department at the Weizmann Institute of Science, Rehovot, Israel.

Below 1 K

Refrigeration and Thermometry below One Kelvin. By D. S. Betts. Pp. x + 283. (Sussex University: Sussex, 1976.) £8.00.

ONE of the most surprising things about research at very low temperatures, to the uninitiated, is its continuing interest and richness. With each further improvement in technology, enabling physicists to push even closer to their unattainable goal of the absolute zero of temperature, there have emerged new and fascinating phenomena to be observed, quantified, and pondered about. The most significant area of technical development over the past decade has undoubtedly been that of ³He-⁴He dilution refrigeration, which has now reached the point where commercially available machines are capable of maintaining a sample indefinitely at temperatures near 10 mK, thus providing a convenient starting temperature from which the 1 mK region becomes immediately accessible through the addition of, for example, an adiabatic demagnetisation stage. Near 1 mK, of course, are the extraordinarily complex 'new phases' of liquid ³He which, with their superfluidity, magnetism and anisotropy, will continue as a challenge to the physicist

for many years to come. At still lower temperatures, in the as yet virtually unexplored region around 0.1 mK, there is the beckoning prospect of nuclear ferromagnetism.

Dr Betts' book, explaining how to do the experiments, is intended as an introduction for MSc and PhD students, and other beginners whose research involves making measurements below 1 K. The first half of the book describes how to achieve these low temperatures, and consists of six chapters covering ³He and ⁴He evaporators, ³He-⁴He dilution refrigerators, Pomeranchuk cooling, and the adiabatic demagnetisation of paramagnetic salts and nuclei. Less popular techniques such as vortex refrigeration have (not unreasonably) been omitted. Having produced a low temperature the next problem is, of course, that of measuring it. Accordingly, the second half of the book is devoted to a detailed survey of the numerous methods which are available, involving measurements of vapour pressure, paramagnetic susceptibility, nuclear magnetic resonance, electrical resistance, capacitance, thermoelectric e.m.f., electrical noise, the Mössbauer effect, and anisotropic radioactive decay.

The various techniques are outlined with impressive clarity, including a useful amount of mathematical detail, and there are copious references both to review articles and also to the original literature. To keep one's feet firmly on the ground, the book is permeated with valuable numerical information both in graphical and in tabular form, the extensive compilation of data on commonly used paramagnetic salts being a particularly striking example. The index is skimpy, but the careful and logical arrangement of material means that elusive topics can usually be traced fairly rapidly by consulting the table of contents. There is inevitably a substantial overlap in coverage with O. V. Lounasmaa's *Experimental Principles and Methods Below 1 K* (Academic, London 1974.) Quite apart from its somewhat different style and emphasis, however, Dr Betts' book has the advantage of being two years more up to date, a factor not to be ignored in this rapidly developing field.

The book succeeds admirably in its stated purposes. It will also be a valuable source of reference for everyone working below 1 K, and most especially for those aspiring to join, in Dr Betts' phrase, "the new army of nuclear coolers... marching into the submillikelvin region."

P. V. E. McClintock

Dr McClintock is a Lecturer in Physics at the University of Lancaster, UK.

Genes or environment

Heredity, Environment, and Personality: A Study of 850 Sets of Twins. By John C. Loehlin and Robert C. Nichols. Pp. xii+202. (University of Texas: Austin, Texas and London, 1976.)

MODEST numbers of twins, carefully sampled and personally investigated in clinical detail, sometimes over a period of years, have an acknowledged place as a valuable if limited research method, particularly in the field of psychiatric abnormality. Attention has recently been drawn, however, to the need for large samples for the adequate study of genetic and environmental influences on ability, personality and interests in the normal population. The present study reports data on some 2,000 variables collected from 850 pairs of twins, not one of whom was personally seen by the authors. The information was obtained from examination results and from questionnaires sent by post to the twins and their parents.

The subjects were those twins who participated in the National Merit Scholarship Qualifying Test taken each year by about 600,000 American high school students at the age of 16 or 17 and who completed the postal questionnaires. The authors clearly discuss the effect of possible biases caused by diagnosis of zygosity by questionnaire, selection for academic ability and willingness to cooperate, and the usual excess of identicals and females found in such samples.

They present data and discuss differences between twins and non-twins, and examine in detail early environmental experiences such as dressing alike, parents treating the twins alike, and interpersonal relationships. None of these had much, if any, influence on the resemblance found. The authors conclude that the environment "operates in remarkably mysterious ways, given traditional views on personality and motivational development".

Not surprisingly, identical twins were no more alike than fraternal twins in matters such as race—or their opinions about racial integration. In most measures, however—whether single items, clusters, or the scales of a test such as the California Psychological Inventory—identicals were usually correlated about 0.20 higher than fraternal twins. On ability measures, intrapair correlations clustered around 0.75 and 0.55 in the two kinds of twins, personality measures, 0.50 and 0.30, and measures of self-concept and interests, 0.35 and 0.15.

The authors' main conclusion, that the almost ubiquitous genetic influence is roughly equal in degree for all traits, is admittedly controversial. Furthermore, some of the statistical analyses require critical interpretation, and little attention is given to which traits are influenced by the same genes. Useful appendices presenting the questionnaires and the intraclass correlations for the twins on each variable are fertile soil for the interested twin researcher. The book can be recommended for its lucid, readable presentation and discussion of the authors' own data and the relevant literature.

**Gregory Carey
James Shields**

Gregory Carey, of the Behavior Genetics Center, University of Minnesota, Minneapolis, Minnesota, is at present a Research Worker at the Institute of Psychiatry, London, UK. James Shields is Reader in Psychiatric Genetics at the same Institute.

Insect clocks

Insect Clocks. (International Series in Pure and Applied Biology. Division: Zoology, Vol. 54.) By D. S. Saunders. Pp. viii+279. (Pergamon: Oxford and New York, July 1976.) £9.25.

THE study of biological clocks is one of those areas of the biosciences that truly straddles the plant and animal kingdoms. The existence of clock-like processes is apparently universal in eukaryotic life, and the clocks of phylogenetically widely separated species exhibit remarkably similar characteristics. Whether this indicates evolutionary convergence or a common, primitive root is not clear, but what it does indicate is why the study of biological clocks has been, and must be multidisciplinary.

The need for a monograph on the clocks of insects may therefore not be immediately apparent. Its justification lies in the simple fact that in no other single order of organisms has so much research been done on so many different kinds of clock. Herein lies the strength of *Insect Clocks*: it provides a comprehensive cover of 'chronobiology' (the subject's American name) in a microcosm.

The book starts off on circadian (that is, 24-h) rhythms, in individuals (chapter 2), in populations (chapter 3), and in physiology (chapter 4). This separation is not entirely happy because rhythms exhibit the same characteristics at whatever level of organisation they occur, so phenomena such as temperature compensation, entrainment and phase-response curves have

to be considered three times over, and the treatment, although thorough, is unnecessarily fragmented.

More than half the book, however, is devoted to insect photoperiodism—that is, the reactions of insects to seasonally changing daylength. Here, the author's touch is sure and his exposition and explanation first rate. Biologists of any specialisation wishing to understand the involutions of this tortuous subject could go a long way before finding a more comprehensive or better account. Whether this otherwise admirable explanation is aided by the chronobiologists' addiction to algebraic jargon is another matter. For example, it is simply irritating, rather than informative, to be told eight times in as many pages (8–16) that τ is the symbol for the free running period of rhythms; and does it help to be given the equation $\tau - T = \Delta\phi_{ss}$ by way of parenthetical expansion of the perfectly clear phrase "... entrainment is effected by discrete apparently instantaneous phase-shifts ..." (p38)? There is a great deal of such symbolising, much of it seemingly unnecessary.

The author unashamedly sticks to insects, his self-confessed lifelong passion. Although this single-mindedness provides the book with its satisfying unitary structure, it does afflict it with an element of tunnel vision. This has prevented the author from considering any of the recent exciting work on the fundamental mechanisms of circadian oscillators, presumably because that work concerns plants, protista and molluscs. Very little is offered on the mechanisms of oscillators in general (the word 'membrane' does not appear anywhere in the otherwise full index, for example), and even on insect clock mechanisms the coverage is less thorough than in some recent reviews. The reader looking for guidance on the modern ideas about oscillator mechanisms, or for an account of the comparative physiology of clocks will therefore be disappointed.

This is the book's only serious weakness: the unifying thread woven through the fabric of the text is taxonomic rather than physiological. Nevertheless, it is an excellent source work, particularly on photoperiodism, and is to be welcomed for that reason alone. In addition, it provides a much more general textbook on chronobiology than its narrow title implies, a paradigm for the subject as a whole, and in that respect is a considerable advance on any of its rivals with more pretentious titles.

John Brady

John Brady is Reader in Insect Behaviour in the Department of Zoology and Applied Entomology at Imperial College, London, UK.

announcements

Awards

Royal Society Medals:

A Royal Medal to **J. W. Cornforth**, University of Sussex, for his fundamental contribution to our knowledge of the biosynthesis of steroids.

A Royal Medal to **J. L. Gowans**, University of Oxford and Medical Research Council Cellular Immunology Unit, for his distinguished research in the field of immunology, especially as regards the recirculation and immunological role of lymphocytes.

A Royal Medal to **A. Walsh**, Division of chemical physics, Commonwealth Scientific and Industrial Research Organization, Australia, for his distinguished contributions to emission and infra-red spectroscopy and his origination of the atomic absorption method of quantitative analysis.

The Copley Medal to **Dorothy M. C. Hodgkin**, University of Oxford, for her outstanding work on the structure of complex molecules, particularly penicillin, vitamin B₁₂ and insulin.

The Rumford Medal to **I. Prigogine**, professor and director of the Free University of Brussels, for his distinguished contributions to the theory of irreversible thermodynamics.

The Davy Medal to **R. E. Richards**, Merton College, Oxford, for his outstanding contributions to nuclear magnetic resonance spectroscopy and its application to chemical and biological problems.

The Darwin Medal to **Charlotte Auerbach**, University of Edinburgh, for her discovery of and continuing work on chemical mutagenesis.

The Sylvester Medal to **D. G. Kendall**, University of Cambridge, for his many distinguished contributions to probability theory and its applications.

The Hughes Medal to **S. W. Hawking**, University of Cambridge, for his distinguished contributions to the application of general relativity to astrophysics, especially to the behaviour of highly condensed matter.

Meetings

December 14–16, **Biochemical Society**, 566th Meeting, Cambridge; January 6–7, 1977, 577th Meeting, Durham (Meetings Officer, 7 Warwick Court, Holborn, London WC1).

January 4–5, 1977, **Terpenoid Biochemistry**, London (Dr M. F. Roberts, School of Pharmacy, London Univer-

Person to Person

An appeal has been launched for £40,000 to commemorate Dr Henry Miller, Professor of Neurology and Vice-Chancellor of Newcastle University, who died August 25, 1976. It is planned to found a research post in the Department of Neurology and to give an annual concert or recital. Donations and enquiries to Mr A. Tindall, 12 Windsor Terrace, Newcastle upon Tyne, UK.

Chemist and wife have furnished house in Cambridge: three large bedrooms, well fitted kitchen, gas-fired central heating, garage, quiet location, convenient for University departments, MRC units, Addenbrookes hospital. Wish to exchange for furnished house in or near Williamsburg, Virginia while on sabbatical leave, August 1977–August 1978. Peter Sykes, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, UK.

There will be no charge for this service. Send items (not more than 60 words) to Marcus Dobbs at the London Office. The section will include exchanges of accommodation, personal announcements and scientific queries. We reserve the right to decline material submitted. No commercial transactions.

sity, 29/39 Brunswick Square, London WC1).

January 12, **Dielectrics**, London (Meetings Officer, Institute of Physics, 47 Belgrave Square, London SW1).

January 16, **Radiation and Materials Modification**, London (address as above).

March 23–25, **Radiation Effects in Liquids and Solids**, Leicester (Mrs Y. A. Fish, Chemical Society, Burlington House, London W1).

March 29–31, **Anisotropic Dielectrics**, Cambridge (Dr C. W. Smith, Department of Electrical Engineering, University of Salford).

March 27–April 8, **Electron Microscopy in Biology**, Erice, Sicily (Dr I. R. Pasquali, Istituto di Patologia Generale, Via Campi 287, Modena, Italy).

April 4–6, **Biochemical Aspects of Plant and Animal Coevolution**, Reading (J. B. Harborne, Department of Botany, University of Reading).

May 30–June 3, **Ferritic Steels for Fast Reactor Steam Generators**, London

(Institution of Civil Engineers, 1–7 Great George Street, London SW1).

June 7–9, **Trace Substances in Environmental Health**, Columbia, Missouri (Dr D. D. Hemphill, 411 Clark Hall, University of Missouri, Columbia, Missouri 65201).

June 20–22, **Mass Spectroscopy in Biochemistry and Medicine**, Riva del Garda, Italy (Dr A. Frigerio, Istituto de Ricerche Farmacologiche, Via Eritrea, 62, Milan, Italy).

July 11–18, **Physical Chemistry and Hydrodynamics** (Levich 60th Birthday Conference), Oxford (D. B. Spalding, Imperial College, Exhibition Road, London SW7).

July 25–29, **High Pressure Applications and Techniques**, Boulder, Colorado (K. D. Timmerhaus, Engineering Research Center, University of Colorado, Boulder, Colorado 80302).

July 26–28, **Synthesis in Organic Chemistry**, Oxford (J. F. Gibson, The Chemical Society, Burlington House, London W1).

September 5–8, **Acetylenes, Allenes and Cumulenes**, Nottingham (address as above).

September 19–23, **Biological Oxidation of Nitrogen in Organic Molecules**, London (J. W. Gorrod, Chelsea College, 271 King Street, London W6).

September 26–29, **Heterogeneous Oxidation**, Leeds (J. F. Gibson, Chemical Society, Burlington House, London W1).

October 24–28, **Water Chemistry of Nuclear Reactor Systems**, Bournemouth, England (Institution of Civil Engineers, 1–7 Great George Street, London SW1).

November 14–17, **Pineal Organ**, Jerusalem (I. Nir, Faculty of Medicine, Hadassah Medical School, Jerusalem).

November 28–December 1, **Optimisation of Sodium-cooled Fast Reactors**, London (Institution of Civil Engineers, 1–7 Great George Street, London SW1).

December 12–14, **Monitoring of Hazardous Gases in the Working Environment**, London (J. F. Gibson, Chemical Society, Burlington House, London W1).

May 13–19, 1979, **Radiation Research**, Tokyo (S. Okada, Department of Radiation Biophysics, Faculty of Medicine, University of Tokyo, Hongo, Tokyo, Japan).

Recent scientific and technical books

Physics

- FULLER, Everett G., and HAYWARD, Evans (edited by). *Photonuclear Reactions*. (Benchmark Papers in Nuclear Physics/2.) Pp.xviii+426. ISBN-0-470-15142-0. (Stroudsburg, Pennsylvania: Dowden, Hutchinson and Ross, Inc., 1976. Distributed by Halsted Press, a Division of John Wiley and Sons, Inc., New York and London.) \$38; £21.
- KILLEEN, John. *Controlled Fusion*. (Methods in Computational Physics: Advances in Research and Applications, Vol. 16.) Pp.xiii+450. ISBN-0-12-460816-7. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1976.) \$47; £27.25.
- KESSLER, Joachim. *Polarized Electrons* (Texts and Monographs in Physics.) Pp.ix+223. ISBN-3-540-07678-6. (Berlin and New York: Springer-Verlag, 1976.) DM 59.80; \$24.60.
- LANDOLT-BÖRNSTEIN, Numerical Data and Functional Relationships in Science and Technology/Zahlenwerte und Funktionen aus Naturwissenschaften und Technik. New Series/Neue Serie. Editor-in-Chief: K. H. Hellwege, Group 2: Atomic and Molecular Physics/Atom- und Molekularphysik. Vol. 7: Structure Data of Free Polyatomic Molecules/Strukturdaten freier Mehratomiger Moleküle. By J. H. Callomon, E. Hirota, K. Kuchitsu, W. J. Lafferty, A. G. Maki and C. S. Pote. With the assistance of I. Buck and S. Starck. Edited by K. H. Hellwege and A. M. Hellwege. Pp.vii+395. ISBN-3-540-07577-1. (Berlin and New York: Springer-Verlag, 1976.) DM 360; \$147.60.
- LANDOLT-BÖRNSTEIN, Numerical Data and Functional Relationships in Science and Technology/Zahlenwerte und Funktionen aus Naturwissenschaften und Technik. New Series/Neue Serie. Editor-in-Chief: K. H. Hellwege, Group 2: Atomic and Molecular Physics/Atom- und Molekularphysik. Vol. 8 (Supplement to Volume 2/Ergänzung zu Band 2. Magnetic Properties of Coordination and Organometallic Transition Metal Compounds/Magnetische Eigenschaften der Koordinations- und Metallorganischen Verbindungen der Übergangselemente, Ergänzungsband 1 (1964-1968). Editors: K. H. Hellwege and A. M. Hellwege. Pp.xxxvii+1163. ISBN-3-540-07441-4. (Berlin and New York: Springer-Verlag, 1976.) DM 1100; \$451.
- MEHRING, M. *High Resolution NMR Spectroscopy in Solids*. (NMR: Basic Principles and Progress/Grundlagen und Fortschritte, Vol. 11.) Pp.xi+246. ISBN-3-540-07704-9. (Berlin and New York: Springer-Verlag, 1976.) DM 68; \$27.90.

Chemistry

- FULLER, C. W. (edited by). *Annual Report on Analytical Atomic Spectroscopy: Reviewing 1975*. Vol. 5. Pp.viii+267. ISBN-0-85186-757-X. (London: The Chemical Society, 1976.) £15; \$33.75.
- HEILBRONNER, Edgar, and BOCK, Hans. *The HMO Model and its Application*. Vol. 2: Problems with Solutions. Translated by William Martin and Anthony J. Rackstraw. Pp.viii+449. ISBN-0-471-01473-7. (London and New York: Wiley-Interscience, John Wiley and Sons; Weinheim: Verlag Chemie, GmbH., 1976.) £15; \$33.00.
- HEILBRONNER, Edgar, and BOCK, Hans. *The HMO Model and its Application*. Vol. 3: Tables of Hückel Molecular Orbitals. Translated by William Martin and Anthony J. Rackstraw. Pp.190. ISBN-0-471-01474-5. (London and New York: John Wiley and Sons; Weinheim: Verlag Chemie, GmbH., 1976.) £9; \$18.
- KEITH, Lawrence H. (edited by). *Identification and Analysis of Organic Pollutants in Water*. Pp.x+718. ISBN-0-250-40131-2. (Ann Arbor, Michigan: Ann Arbor Science, Publishers, Inc.; Chichester: John Wiley and Sons, Ltd., 1976.) \$30.25; £17.90.
- PEARSON, Ralph G. *Symmetry Rules for Chemical Reactions: Orbital Topology and Elementary Processes*. Pp.ix+548. ISBN-0-471-01495-8. (New York and London: Wiley-Interscience, John Wiley and Sons, 1976.) \$31; £17.50.

Technology

- BLASCHKE, W. S., and MCGILL, J. *The Control of Industrial Processes by Digital Techniques: The Organization, Design and Construction of Digital Control Systems*. Pp.viii+185. ISBN-0-444-41493-7. (Amsterdam, Oxford and New York: Elsevier Scientific Publishing Company, 1976.) Dfl. 77; \$29.75.
- EVAN-IEWANOWSKI, R. M. *Resonance Oscillations in Mechanical Systems*. Pp.xi+292. ISBN-0-444-41474-6. (Amsterdam, Oxford and New York: Elsevier Scientific Publishing Company, 1976.) Dfl. 77; \$29.75.
- FLOWERS, T. H. *Introduction to Exchange Systems*. Pp.xvii+326. ISBN-0-471-01865-1. (London and New York: Wiley-Interscience, John Wiley and Sons, 1976.) £13; \$26.
- GOLARD, R. (edited by). *Combustion Measurements: Modern Techniques and Instrumentation*. (A Project Squid Workshop.) Pp.xii+483. ISBN-0-12-294150-0. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers; Washington and London: Hemisphere Publishing Corporation, 1976.) \$34.50; £21.05.
- JACOBSON, Kurt I., and JACOBSON, Ralph E. *Imaging Systems: Mechanisms and Applications of Established and New Photosensitive Processes*. Pp.319. (London and New York: The Focal Press, 1976.) £11.95 net.
- MADDIN, Robert (editor-in-chief). *Challenges and Opportunities in Materials Science and Engineering*. (Anniversary volume of *Materials Science and Engineering*.) Pp.vi+264. (Lausanne: Elsevier Sequoia, S. A., 1976.)
- MENDELSSOHN, K. (edited by). *Proceedings of the Sixth International Cryogenic Engineering Conference*, Grenoble, 11-14 May 1976. (ICEC, 6.) Pp.827. ISBN-0-902852-58-2. (Guildford: IPC Science and Technology Press, Ltd., 1976.) £29.
- MCDUGALL, Angus. *Fuel Cells*. (Energy Alternatives Series.) Pp.xii+147. ISBN-333-18408-4. (London and Basingstoke: The Macmillan Press, Ltd., 1976.) Hard cover £7.95; Paper cover £2.95.
- PALMER, Andrew C. *Structural Mechanics*. (Oxford Engineering Science Texts.) Pp.x+218. ISBN-0-19-856128-8. (Oxford: Clarendon Press; London: Oxford University Press, 1976.) Paper £3.50 net; Boards £7 net.
- ROBERTSON, J. Craig. *A Guide to Radiation Protection*. Pp.86. ISBN-333-19278-8. (London and Basingstoke: The Macmillan Press, Ltd., 1976.) £4.95.
- ROBERTSON, Angus (edited by). *Video Yearbook 1977*. Pp.286. ISBN-0-85642-064-6. (Poole: Dolphin Press, an imprint of Blandford Press, 1976.) £4.75.
- RUSSELL, Clifford S., and VAUGHAN, William J. *Steel Production: Processes, Products, and Residuals*. Pp.xx+328. ISBN-0-8018-1824-9. (Baltimore and London: The Johns Hopkins University Press, 1976. Published for Resources for the Future.) £12.40.
- SZEKELY, Julian, EVANS, James W., and SOHN, Hong Yong. *Gas-Solid Reactions*. Pp.xiii+400. ISBN-0-12-680850-3. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1976.) \$39.50; £24.10.

Earth sciences

- GOLDBERG, Edward D. *Strategies for Marine Pollution Monitoring*. Pp.x+310. ISBN-0-471-31070-0. (New York and London: Wiley-Interscience, John Wiley and Sons, 1976.) \$27.50; £16.50.

Biological sciences

- ANDERSON, J. M., and MADFADYEN, A. (edited by). *The Role of Terrestrial and Aquatic Organisms in Decomposition Processes*. (The 17th Symposium of The British Ecological Society, 15-18 April 1975.) Pp.xi+474. ISBN-0-632-00018-X. (Oxford and London: Blackwell Scientific Publications, 1976.) £14.

- BAKER, Joseph T., and MURPHY, Vreni. *Handbook of Marine Science*. Vol. 1: Compounds from Marine Organisms. Pp.228. ISBN-0-87819-388-X. (Cleveland, Ohio: CRC Press, Inc., 1976.) \$29.95.
- BALTIMORE, David, HUANG, Alice S., and FOX, C. Fred (edited by). *Animal Virology*. (ICB-UCLA Symposia on Molecular and Cellular Biology, Vol. IV, 1976.) Pp.xi+824. ISBN-0-12077350-3. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1976.) \$29.50; £18.
- BANKS, P., BARTLEY, W., and BIRT, L. M. *The Biochemistry of the Tissues*. Second edition. Pp.xv+493. ISBN-0-471-05471-2. (London and New York: John Wiley and Sons, 1976.) Cloth £14.50; \$30. Paper £6.26; \$13.50.
- BROWN, Leslie. *Eagles of the World*. Pp.224. ISBN-0-7153-72696 (Newton Abbot and London: David and Charles, 1976.) £4.95.
- BROWN, Leslie. *Birds of Prey: Their Biology and Ecology*. Pp.256. ISBN-0-600-31306-9. (London and New York: Hamlyn Publishing Group, Ltd., 1976.) £4.50.
- CAPUTO, A. (edited by). *Biological Basis of Clinical Effect of Bleomycin*. (Progress in Biochemical Pharmacology, Vol. 11.) Pp.x+230. ISBN-3-8055-2338-6. (Basel, London and New York: S. Karger, 1976.) SFr./DM 127.
- CHAPMAN, V. J. *Coastal Vegetation*. Second edition. (Pergamon International Library of Science, Technology, Engineering and Social Studies.) Pp.viii+292. ISBN-0-08-020896-7. (Oxford and New York: Pergamon Press, 1976.) Hard case £8.75; Flexicover £4.75.

History of science

- BRIEGER, Gert H. (edited by). *Theory and Practice in American Medicine: Historical Studies from the Journal of the History of Medicine and Allied Sciences*. Pp.xv+272. (New York: Science History Publications, a Division of Neale Watson Academic Publications, Inc., 1976.) Cloth \$12; Paper \$4.95.
- COLLARD, Patrick. *The Development of Microbiology*. Pp.201. ISBN-0-521-21177-8. (Cambridge and London: Cambridge University Press, 1976.) £6.50.
- COPERNICUS, On the Revolutions of the Heavenly Spheres. A New Translation from the Latin with an introduction and notes by A. M. Duncan. Pp.328. ISBN-0-7153-6927-X. (Newton Abbot and London: David and Charles; New York: Barnes and Noble Books, a Division of Harper and Row, Publishers, 1976.) £12.50.
- GIFFORD, Jr., George E. (edited by). *Physician Signers of the Declaration of Independence*. Pp.163. ISBN-0-88202-159-1. (New York: Science History Publications, a Division of Neale Watson Academic Publications, Inc., 1976.) \$10.
- KANGRO, Hans. *Early History of Planck's Radiation Law*. Pp.xvii+282. ISBN-0-85066-063-7. (London: Taylor and Francis, Ltd., 1976.) £25.
- MCCORMACH, Russell (edited by). *Historical Studies in the Physical Sciences*. Seventh Annual Volume. Pp.xxxv+489. ISBN-0-691-08169-7. (Princeton, NJ: Princeton University Press, 1976.) £19.80.

Anthropology

- BAKER, Paul T., and LITTLE, Michael A. (edited by). *Man in the Andes: A Multidisciplinary Study of High-Altitude Quechua*. (US/IBP Synthesis Series 1.) Pp.xx+482. ISBN-0-87933-228-X. (Stroudsburg, Pennsylvania: Dowden, Hutchinson and Ross, Inc., 1976. Distributed by Halsted Press, a Division of John Wiley and Sons, Inc., New York and London.) £18; \$30.

Psychology

- BARBER, T. X. *Pitfalls in Human Research: Ten Pivotal Points*. (Pergamon General Psychology Series.) Pp.vii+117. ISBN-0-08-020935-1. (Oxford and New York: Pergamon Press, 1976.) \$6.95.
- JUNG, C. G. *Analytical Psychology: Its Theory and Practice*. (The Tavistock Lectures.) Pp.xvi+224. ISBN-0-7100-8414-5. (London and Henley: Routledge and Kegan Paul, Ltd., 1976.) Paperback £1.95.
- LIPSTITZ, Lewis P. (edited by). *Developmental Psychobiology: The Significance of Infancy*. Pp.x+143. ISBN-0-470-15127-7. (Hillsdale, NJ: Lawrence Erlbaum Associates, Publishers, 1976. Distributed by Halsted Press, a Division of John Wiley and Sons, New York and London.) \$12.65; £7.65.
- MEDLIN, Douglas L., ROBERTS, William A., and DAVIS, Roger T. (edited by). *Processes of Animal Memory*. Pp.xi+267. ISBN-0-470-15189-7. (Hillsdale, NJ: Lawrence Erlbaum Associates, Publishers, 1976. Distributed by the Halsted Press Division of John Wiley and Sons, New York and London.) \$19; £11.50.
- NORMAN, Donald A. *Memory and Attention: An Introduction to Human Information Processing*. (Series in Psychology.) Second edition. Pp.xiii+262. ISBN-0-471-65136-2. (New York and London: John Wiley and Sons, Inc., 1976.)
- PORGES, Stephen W., and COLES, Michael G. H. (edited by). *Psychophysiology*. (Benchmark Papers in Animal Behavior/6.) Pp.xv+366. ISBN-0-470-15136-6. (Stroudsburg, Pennsylvania: Dowden, Hutchinson and Ross, Inc., 1976. Distributed by Halsted Press, a Division of John Wiley and Sons, Inc., New York and London.) \$32; £17.75.

Sociology

- BLACK, Donald. *The Behavior of Law*. Pp.xi+175. ISBN-0-12-102650-7. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1976.) \$12.50; £7.60.
- CHIROT, Daniel. *Social Change in a Peripheral Society: The Creation of a Balkan Colony*. (Studies in Social Discontinuity.) Pp.xvii+179. ISBN-0-12-173150-2. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1976.) \$12.50; £7.25.
- KORNITZER, Margaret. *Adoption*. Pp.186. Fifth edition. ISBN-0-370-10059-X. (London: Putnam and Company, 1976.) Hardback £4.95; Paperback £3.50.
- McKEOWN, Thomas. *The Modern Rise of Population*. Pp.168. ISBN-0-7131-5867-0. (London: Edward Arnold (Publishers), Ltd., 1976.) £7.95.
- ROSE, Hilary, and ROSE, Steven. *The Radicalisation of Science: Ideology of/in the Natural Sciences*. (Critical Social Studies.) Pp.xxvi+205. ISBN-0-333-21141-3. (London and Basingstoke: The Macmillan Press, Ltd., 1976.) Hard cover £10; Paper cover £3.95.
- ROSE, Hilary, and ROSE, Steven (edited by). *The Political Economy of Science: Ideology of/in the Natural Sciences*. (Critical Social Studies.) Pp.xxvi+218. ISBN-0-333-21139-1. (London and Basingstoke: The Macmillan Press, Ltd., 1976.) Hard cover £10; Paper cover £3.95.

Geography

- OEHSER, Paul H. (compiled and edited by). *Research Reports*. (Abstracts and reviews of research and exploration authorized under grants from the National Geographic Society during the year 1968.) Pp.x+527. ISBN-087044-136-1. (Washington, DC: National Geographic Society, 1976.) \$5.

General

- BUSVINE, J. R. *Insects, Hygiene and History*. (London: The Athlone Press, University of London, 1976. Distributed by Tiptree Book Services, Ltd., Tiptree, Essex: Humanities Press, Inc., New Jersey.) £6.95.
- FARSON, Daniel (compiled by). *In Praise of Dogs*. Pp.118. (London: George G. Harrap and Co., Ltd., 1976.) £3.95 net.
- GOLDSTEIN-JACKSON, Kevin. *Experiments With Everyday Objects*. Pp.187. ISBN-0-285-62245-5. (London: Souvenir Press, Ltd., 1976.) £3.
- ROSZAK, Theodore. *Unfinished Animal: The Aquarian Frontier and the Evolution of Consciousness*. Pp.ix+271. ISBN-0-571-11014-2. (London: Faber and Faber, Ltd., 1976.) £2.95.